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ABSORPTION OF POTASSIUM BY PLANTS IN RELATION TO REPLACEABLE, NON-REPLACEABLE, AND SOIL SOLUTION POTASSIUM

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It is still very uncertain what general principles or methods of study are applicable when problems of potassium nutrition arise in connection with hitherto uninvestigated soil and crop conditions. Among the points needing further consideration, the following may be suggested: (*a*) physiological importance of potassium held in the base exchange complex of soils relative to other forms of potassium; (*b*) fixation of potassium in non-replaceable form; (*c*) extent to which displaced soil solutions or aqueous extracts of soils reflect physiological conditions with regard to potassium; (*d*) importance of the fixation of potassium by soils in connection with the fertilization of deep-rooted crops; (*e*) availability of potassium fixed in different ways, under favorable conditions for root contacts; (*f*) reliability of data on composition of crop as reflecting availability of potassium in soils; (*g*) relation between the "luxury" absorption of potassium by crops and ascertainable chemical conditions in soils. These are not new questions, but present knowledge remains inadequate.

The incentive for the present investigation was not wholly academic in nature. Certain practical problems had arisen, among which was a disease of prune trees found in certain districts of California. Potassium deficiency was discovered to be one of the important causal factors in this disease. However, this particular problem, although it has been intensively investigated for the past six years by the divisions of pomology and of plant nutrition, is not the subject of this report. The plant culture studies to be described deal mainly with barley and tomato plants as indicators of physiological conditions existing in the soil. The primary objective was to gain some additional general understanding of plant growth in relation to the supplying power of soils for potassium.

CHEMICAL METHODS APPLIED TO SOILS

It was first essential to consider the various forms, including the replaceable form, in which K is held in soils. Unfortunately, there exists no method by which replaceable K can be accurately differentiated from that entering into solution from minerals not involved in simple base replacement. When the amount recovered by leaching with an ammonium salt[2] is relatively very small, as is often the case, a large percentage may not be assignable to typical replacement processes. On the other hand, when a soil yields on leaching with the reagent a relatively large amount of K, even though the absolute amount may be very small, it is probable that the major portion entered into solution as a result of replacement. In the soils investigated by us, the fraction leached from a soil by the given technique could be rather sharply defined, inasmuch as additional leaching usually yielded only very minute amounts of K. In certain soils recently studied by Wheeting (22) in Wisconsin, a somewhat different type of behavior was observed. Most of the values given in this paper for replaceable K include also water-soluble K. The latter forms a very small proportion of the total in soils of high fixing power. In special cases, the water-soluble K is deducted from the total as determined by leaching with ammonium acetate.

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[1] The extensive laboratory and greenhouse experiments involved in this investigation could not have been performed without the able assistance of Messrs. R. Overstreet, P. R. Stout, A. H. Furnstal, and others. We are also indebted to the American Potash and Chemical Company for a grant in aid of some phases of the research.

Serious, and not always recognized, analytical difficulties may be met with in the determination of K in the leachate of a soil made with ammonium salts. Many soils yield very large amounts of Ca and Mg and extremely small amounts of K. On the general question of K determinations in soils, Milne (16), in a recent article, suggests that gross errors may conceivably be resident in much of the extant data on soil potassium as determined by the usual technique of the platinic chloride method. In our earlier work, this method was employed after the separation of Ca and Mg. Even when all possible care was taken, many results seemed to be seriously in error. A special study was therefore made of analytical technique. The method finally adopted included the preliminary separation of K as the cobalti-nitrite, and its final precipitation as the chloroplatinate. More recently, success has been attained by several workers in the laboratory with a titration procedure applied to the cobalti-nitrite precipitate. Apparently the latter method is capable of giving accurate results but involves certain details of manipulation not controlled with equal success by all analysts. Other procedures employed in studying soils were similar to those heretofore described in the literature.

SAMPLING OF SOIL

Errors of soil sampling also require careful consideration. Even when the amount of K salt added to a soil is of the magnitude of a very heavy fertilizer application, still the proportion of salt to soil is exceedingly small, and it is correspondingly difficult to obtain from a large lot of soil an analytical sample representing an average condition. This difficulty we have verified by specific studies. The conclusion is that the most accurate study of the fate of K added to a soil must be made by a special laboratory technique. In this technique, suitable portions of soil are weighed out and to these portions are added accurately weighed amounts of a K salt, the whole portion of soil being used in the extraction process. Thus it is known for each sample exactly how much K has reacted with a given amount of soil. To investigate the effect of cropping, it is, of course, inevitable that samples be taken from fairly large lots of soil. The only recourse in this case is to utilize great precaution in taking the samples. In the more crucial experiments, we have adopted the procedure of taking 30 cores of soil from each crock, followed by repeated mixing and quartering until finally a sample of about 300 gm. is obtained.

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[2] In certain cases, comparisons were made between the amounts of K removed by ammonium acetate and by 0.05 *N* HCl. The agreement between the two methods was close. For further discussion of methods for studying base replacement, with special reference to arid soils, see Chapman and Kelley (5).

Potassium not extracted by the ammonium acetate procedure is regarded as non-replaceable. It is not considered that the use of the terms "intensively" and "extensively" exchangeable potassium, as suggested by Gedroiz (7), would add to the clarity of the discussion. Non-replaceable potassium may enter into solution accompanied by silica, or through processes of slow hydrolysis.

FIXATION OF POTASSIUM IN NON-REPLACEABLE FORM BY SOILS

At the time the work was begun, it was assumed that all K added to a soil would be recoverable by leaching the soil with ammonium acetate. Various statements in the literature, usually based on field data, concerning fixation in non-replaceable form, led to the inference that such fixation would generally be slow and gradual. It soon became apparent that certain of the soils studied in this laboratory were capable of fixing significant amounts of K in a non-replaceable form very rapidly, perhaps immediately. In a few instances, the major part of the K added was so fixed, the amounts involved corresponding to a range of light to very heavy fertilizer applications. During the progress of these experiments, the report of Sears (20) was published. Incidental to a general investigation of an infertile Illinois soil, Sears found that this soil was capable of fixing large amounts of K in non-replaceable form. The Illinois soil contained high amounts of $CaCO_3$, but our results show that such fixation may occur in soils not containing appreciable amounts of carbonate. In connection with the question of fixation of bases in non-replaceable form, the views of MacIntire (13) published over a long period should also be kept in mind.

The suggestion may possibly occur that the addition of K to a soil forces back the solution of K derived from resistant soil minerals, so that the amount recovered from a treated soil should be less than the sum of the amount of added K plus the amount of K recovered from the untreated soil. It is not expected that such an effect would be of large magnitude when a drastic leaching process is involved, and in any event this suggestion is entirely inadequate to explain the results, since in some of the treatments the amount of non-recoverable K was much greater than the total amount of K recovered from the untreated soil. Furthermore, in certain soils all the K added was recovered, making allowance for the K leached from the untreated soil. Among the soils giving complete recovery of K, within limits of error, was one of the heaviest soils, whereas several of the lighter soils showed definite fixation of K in non-replaceable form. The data in support of these statements will be presented in another article.

Practically equivalent displacement of other bases occurred even when a large fraction of the added K was fixed in a non-replaceable form. It has not been shown thus far that fixation of K in non-replaceable form is accompanied by loss of total exchange capacity as measured by ammonium absorption, although it remains uncertain whether present methods for determining exchange capacity are capable of yielding values sufficiently accurate to enable a decision to be reached. It is conceivable that the exchange of bases occurring is in part indirect and involves compounds other than the base exchange complex. One may, perhaps, simply say that in effect an irreversible exchange with K takes place. Experiments are also being made to determine whether drying a soil after treatment with a K salt influences the amount of K fixed in non-replaceable form. Certain data suggest that such effects require study. It should be noted that we are not now attempting to distinguish between mineral and organic complexes in connection with the problems of fixation.

Bartholomew and Jannsen (2) concluded that addition of a K salt to an acid soil may cause a greater increase in the amount of replaceable K than can be accounted for by the K actually added to the soil. They suggest that H ions may be liberated from the base exchange complex and that these H ions may dissolve K from resistant soil minerals, the K so dissolved then being fixed in replaceable form. It is difficult to understand this conclusion, since Bartholomew and Jannsen determined the amount of replaceable K by leaching the soil with 0.05 *N* acid. One would suppose that the H ions from the acid used in leaching would be much more effective in dissolving potassium than the H ions liberated following the addition of a salt, in any of the amounts used. None of the soils investigated by us, including one distinctly acid soil, have behaved in the manner described.

RELATION BETWEEN REPLACEABLE AND SOIL SOLUTION POTASSIUM

The relation between soil solution concentrations of K and amounts of replaceable K is of much importance in any consideration of the absorption of K by plants. In a given soil fertilized with different increments of K, concentrations present in the soil solution many be correlated with amounts of K added to the soil and with amounts of replaceable K (fig. 4) but the suggestion of Magistad (14) that the amount of repleaceable K present in a soil provides a general basis for computing concentrations maintained in the soil solution is not upheld by our results. In comparing different soils, much more consistent relative values for the soil solution may be obtained on the basis of the proportion of K to total exchange capacity rather than on that of the absolute amount of replaceable K (unpublished data). But even the degree of saturation for K does not necessarily supply adequate information for accurate estimation of soil solution values when different soils are compared. The types of colloids and of other minerals present cannot be expected to be identical in all soils.

CONDITIONS OF PLANT CULTURE EXPERIMENTS

With these necessary preliminary observations on the relations of K to the soil system in mind, we proceed to a discussion of absorption of K by crops. Barley and tomato plants[3] were found to be well suited to the purposes in view. Characteristic leaf injury of tomato plants reliably reflected deficiencies of supplying power in the culture media under greenhouse conditions. Plants grown in deficient soils were affected in the same manner as plants grown in certain deficient artificial culture solutions [for photograph illustrating type of injury, see Johnston and Hoagland (9)]. Barley plants were not found to display leaf symptoms as characteristic and as certainly recognizable as those just described, but in some instances, the effect of K fertilization on yield was enormous.

Plants were grown in the greenhouse at various seasons of the year, the tomato plants usually during the warmer months. Cultures were replicated three to five times, 2- or 5-gallon containers being employed. Distilled water was used and drainage prevented. Under suitable soil conditions, large and thrifty plants were obtained. The variability in the cultures was generally relatively small, the mean deviation being of the order of 5 to 10 per cent. The number of replicates was insufficient for formal statistical evaluation of yields but this does not seem to be of importance at this stage of development of the investigation, since the differences on which our main contentions are based are so extreme as to be obviously significant, either as regards yield or symptoms of leaf injury, or both. It is also to be noted that one of the primary purposes of this paper is to discuss quantitatively determined withdrawals of K from given masses of soil, rather than yields of crop as such.

A group of 15 different soils was chosen for these studies, including soils of widely varying texture collected from different regions of California. Several of the soils were of decidedly acid or alkaline reaction. The soils employed were more fortunately chosen than might have been anticipated, since several characteristically different types of behavior with regard to K availability were clearly manifested. Some of the soils were selected because of nutritional difficulties occurring with prune or other crops, under field conditions. Others were considered to be soils of high fertility. Soil 30 was similar to a soil which has been under study since 1915, in controlled crop experiments by Burd and Martin (4). The general characteristics of the soils are given in table 1.

FIXATION OF POTASSIUM BY SOILS IN RELATION TO ITS ABSORPTION BY PLANTS

Soil 37 showed an especially marked deficiency in ability to supply K to plants (barley, tomatoes, corn, alfalfa, prune trees, and other plants), and also possessed a very striking power of fixation. After addition of soluble K salts, even in amounts far exceeding those used in ordinary fertilizer practice, 97 to 99 per cent of the added K did not appear in the displaced soil solution. Evi-

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[3] Variety 4,000 and later Atlas variety of barley and Santa Clara Canner variety of tomato in all except one experiment. Barley was grown to maturity, tomatoes to the flowering stage.

TABLE 1

General classification and chemical characteristics of soils used

SOIL NUMBER	CLASSIFICATION	ORIGIN	pH*	REPLACEABLE K IN SOIL	TOTAL K	REPLACEABLE BASES, M. E. PER 100 GM. SOIL						RELATIVE PROPORTION OF BASES, PER CENT OF TOTAL†				FIXING POWER (BASIS OF 1:⅕ H_2O EXTRACT)	
						Ca	Mg.	K	Na	Total†	NH_4 absorbed	Ca	Mg	K	Na	K added to soil	K fixed
				p.p.m.	*per cent*											*p.p.m.*	*per cent*
21D	Oakley blow sand	Oakley	6.4	125	1.83	3.27	0.70	0.31	0.02	4.30	3.66	76.1	16.3	7.2	0.4	85	68
29	Oakdale sandy loam	Modesto	7.9	175	2.26	3.52	0.98	0.45	0.05	5.00	4.24	70.4	19 6	9.0	1.0	155	63
30	Fresno fine sandy loam	Fresno	6.9	140	2.25	4.97	1.32	0.35	0.18	6.82	4.94	72.9	19.4	5.1	2.6	321	61
36	Farwell loam	Chico	6.7	85	0.90	13.98	8.05	0.22	0.36	22.60	23.50	61.8	35.6	1.0	1.6	385	96
37	Nord fine sandy loam‡	Chico	8.2	60	0 60	15.27	15.20	0.15	0.39	31.01	29.75	49.2	49.0	0.5	1.3	396	98
38	Vina clay	Chico	6.7	395	0.56	20.34	6.15	1.01	0.56	28.05	30.00	72.5	21 9	3.6	2.0	390	93
53	Delhi sand	Delhi	7.1	115	2.56	1.64	0.43	0.29	0.22	2.58	2.19	63.6	16.7	11.2	8.5	68	47
64	Vina clay loam	Durham	6.6	70	0.90	15.47	7.97	0.19	0.55	24.18	23.83	64.0	33.0	0.8	2.2	424	97
78	Gold Ridge fine sandy loam	Forestville	4.8	55	0.91	2.76	1.17	0.14	0.15	4.22	5.53	65.4	27.7	3.3	3.6	212	75
80	Hanford sandy loam‡	Porterville	8.3	100	2.02	10.98	2.51	0.25	0.54	14.28	12.44	76.9	17.6	1.8	3.7	189	97
90	Aiken clay	Paradise	5.8	270	0.44	9.12	3.24	0.70	0.08	13.14	17.40	69.4	24.7	5.3	0.6	264	84
95	Ramona loam	Riverside	7.8	60	1.89	5.72	1.76	0.15	0.29	7.92	6.15	72.2	22.2	1.9	3.7	182	97
100	Elder silty loam‡	Hamilton City	7.1	125	1.42	8.43	13.51	0.32	0.52	22.78	20.28	37.0	59.3	1.4	2.3	215	99
101	Yolo clay loam	San Jose	6.7	170	2.01	12.50	3.97	0.44	0.20	17.11	17.35	73.1	23.2	2.6	1.1	202	93
102	Monterey sand	Monterey	6.7	25	1.70	0.50	0.54	0.07	0.16	1.27	1.17	39.4	42.5	5.5	12.6	84	52

* Determined on soil suspension. In majority of cases comparisons were made of quinhydrone, hydrogen, and antimony electrodes. Generally fair agreement was obtained. Values by hydrogen electrode method are given preference where disagreement was found.

† Total of determined bases.

‡ $CaCO_3$ contents of soils 37, 80, and 100 are 1.0 per cent, 0.4 per cent, and 0.2 per cent, respectively; all other soils less than 0.1 per cent.

dently this soil, as well as most of the other soils studied, had a much greater fixing power for K than did the Clarksville loam recently investigated by Bartholomew and Jannsen (2). Several preliminary experiments were made for the purpose of determining to what extent the soil described above could yield to tomato plants K previously fixed following fertilizer applications.

It is, of course, not feasible to differentiate clearly in such experiments between K originally present in a soil and K added to it. Conceivably the addition of a small amount of K to a soil of low supplying power for this element may so increase the extent and activity of the root system as to lead to an augmented draft on the difficultly soluble K naturally occurring in the soil. An effect of opposite tendency will result from the building up of a higher concentration of K in the soil solution as a consequence of fertilization, with a temporary forcing back of the solution of K from certain soil minerals. Since these opposing tendencies cannot be evaluated, we must be content to estimate the differences between the amounts of K absorbed from treated and untreated soils. (Nitrate and phosphate were applied in all cases.) If an untreated soil is capable of supplying to plants only a very small amount of K, then it is reasonable to assume that a useful indication may be obtained of the absorbability of the added K by the method of estimation already referred to. Such a condition seems to obtain in soil 37 and several other soils. Estimates derived from the results of long periods of intensive cropping may be also of value, even for soils of large initial supplying power.

In one of the experiments now referred to, K was applied in several forms, including mixed fertilizer and sheep manure, as well as pure K salt. No certainly significant difference among these carriers was observed with regard to absorption of K by crops. The conclusion from this experiment is that all or nearly all K added to the soil in the smaller increments was absorbed by one or two tomato crops, proving that the almost complete fixation of K by the soil did not make it physiologically unavailable under these particular conditions. It may be noted incidentally that no drying out of the soil occurred during this experiment. In the case of the heavy application of K, the percentage recovery was much lower, but reached approximately 50 per cent. Additional amounts would have been recovered by repeated cropping, as will be shown by later experiments, but it will also be shown that complete recovery is not always attained when large applications of K and long periods of cropping are involved.

RELATION BETWEEN AMOUNTS OF POTASSIUM ADDED TO THE SOIL, AMOUNTS ABSORBED BY CROPS, AND SOIL SOLUTION CONCENTRATIONS

In the next experiment, K salt was added to soil 37 in a series of increments. Five jars of soil were prepared for each treatment, one being left uncropped while four were cropped with tomatoes. At the end of the experiment, the soils were subjected to the displacement procedure of Burd and Martin (4). It is shown by figure 1 that the concentrations of K in the soil solution from the uncropped soil definitely reflect the increasing applications of K salt, notwithstanding the almost complete fixation of K by the soil in question. This is true even of relatively low rates of application, within a range of fertilizer practice. The concentrations of K in the soil solution increase at an increasing rate with successive increments of K added. If the values for soil solution K are plotted logarithmically against amounts of K applied to the soil, a fair approximation to a straight line is obtained. However, even with the heaviest application, only 40 p.p.m. of K are found in the displaced soil solution, over 95 per cent of the added K having been fixed in the solid phase of the soil. The influence of crop growth on concentrations of K in the soil solution is noteworthy. The solid phase components could not keep the soil solution renewed at nearly its original level after the actively growing crop began to assert its full power of absorption.

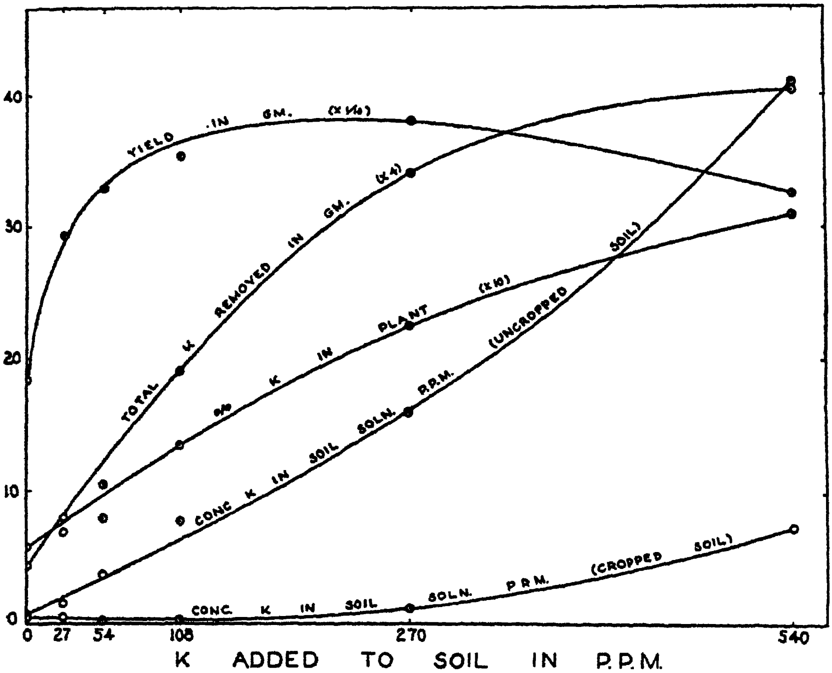

FIG. 1. RELATION BETWEEN COMPOSITION OF SOIL SOLUTION, COMPOSITION OF TOMATO PLANTS, TOTAL POTASSIUM REMOVED FROM SOIL AND INCREMENTS OF POTASSIUM ADDED

Soil 37 used. The treatments correspond approximately to a range of applications of from 230 pounds to 4,600 pounds of sulfate of potassium per acre (area basis).

The line representing the percentages of K in dry plant tissue is approximately parallel to the line representing concentrations of K in the soil solution of the uncropped soil for the first four treatments, but the two lines intersect before reaching the points indicating the largest treatment. The crop had attained approximately the maximum percentage of K for the given environmental conditions and stage of growth. Beyond a certain point, increases in initial concentrations in the soil solution did not, of course, lead to increases in the amount of K absorbed by the crop. The limitation on absorption became physiological. The total amounts of K removed by the crop from a given weight of soil plotted against additions of K follow a logarithmic type of curve. A certainly significant increase in yield is correlated with the first K treatment, but the differences between the three or four highest treatments are of doubtful significance. The plants without treatment showed unmistakable symptoms of K deficiency. Similar symptoms were also displayed by the plants growing in the soil with the lowest application of K, but only during the early stages of growth.

Evidently both the initial and final concentrations of K present in the displaced soil solution could be very low and yet reflect an adequate supplying power. In the second treatment, these concentrations were only 3.6 p.p.m. for the uncropped soil, and 0.3 p.p.m. for the cropped soil. The entire amount of soil solution available at any one time contained as a maximum only a very small percentage of the total K ultimately absorbed by the crop, even in the case of the most heavily fertilized soil. The condition existing in such a soil is, in some respects, not unlike that of a flowing culture solution in which the concentration of K at any one instant is very low. In the soil, the solid phase K forms a reserve for renewing the soil solution; in the flowing culture solution the reserve is found in the large volume of changing solution. In this experiment, magnitudes of the lowest concentrations in the displaced soil solution which reflected a satisfactory condition for the growth of tomato plants, are very similar to those established in earlier solution culture work. Further discussion of this point follows later. With certain of the soil treatments, a large "luxury"[4] consumption of K by the crop took place. The composition of the plant was greatly changed by the higher applications, not only with regard to K content, but also with regard to content of Ca and Mg.

The next step was to compare soil 37 with a soil of low but temporarily adequate supplying power (table 2). Soil 36, from an adjacent field area, produced symptoms of deficiency in tomato plants only after several crops had been grown. Pronounced differences in the appearance and yield of the plants grown in these two soils were clearly evident. The yield of dry matter obtained from soil 36 was several times greater than that from soil 37. The displaced solution from uncropped soil 37, was practically unchanged by cropping. In this soil and in soil 36 the values were very low, but in soil 37 the supplying power for K was below the critical limit, and in soil 36 above this limit. Again,

[4] By this term is meant simply potassium present in the plant tissue at harvest (complete ripeness for barley and blossoming stage for tomato) in excess of that necessary to produce the given yield. Bartholomew and Jannsen have used the term in a slightly different sense to indicate potassium present in excess for an early stage of growth, but essential at a later stage, following translocation.

these soil solution values are of magnitudes not very different from those derived from artificial culture solution experiments.

In both soils, K derived from a non-replaceable form was the chief source of this element for plant growth, but the non-replaceable K in soil 36 had a distinctly greater physiological availability than in soil 37. In each soil, the total amount of K present in the crop as harvested was over 80 times greater than the maximum total amount contained at a given instant in the displaced soil solution of the uncropped soil.

TABLE 2

Absorption of K by tomato plants in relation to soil solution, comparing two low levels of supplying power

SOIL	TREATMENT	H_2O CONTENT AT DISPLACEMENT	COMPOSITION OF SOIL SOLUTION			COMPOSITION OF 1:⅕ EXTRACT			REPLACEABLE K IN DRY SOIL
			Ca	Mg	K	Ca	Mg	K	
		per cent	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p m.*	*p.p.m.*	*p.p.m.*
36	Original	19.8	512	188	4.7	225	101	2.89	96
	After cropping	19.7	274	84	1.4	103	53	0.63	68
37	Original	22.9	360	215	0.9	186	145	0.82	60
	After cropping	22.3	268	134	1.0	141	75	0.25	63

Data on crops

SOIL	YIELD OF CROP*	COMPOSITION OF CROP			TOTAL AMOUNT OF K IN CROP*	LOSS REPLACEABLE K FROM SOIL*	K FROM NON-REPLACEABLE FORM*
		Ca	Mg	K			
	gm.	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*
36	261	1.21	0.57	0.77	2.00	0.70	1.30
37	103	1.83	0.95	0.48	0.49	0	0.49

* Dry weight totals computed on basis of 3 jars for each treatment.

2-gallon jars were used; superphosphate and calcium nitrate applied to all soils.

Period of growth 3–4–30 to 5–19–30. Plants in soil 37 showed foliage injury resulting from K deficiency, while plants in soil 36 remained entirely healthy.

The relation of K to Ca and Mg is of interest in any comparison of soil 36 with soil 37. The latter soil contains about 1 per cent of carbonate and also a large amount of exchangeable or easily acid-soluble Mg. It was thought possible that this high Ca and Mg content, in association with the low supplying power for K, might contribute to the unfavorable physiological condition existing in this soil. This is a possibility, but experiments showed that the addition to soil 36 of calcium carbonate to the extent of 3 per cent, or of magnesium carbonate to the extent of 1 per cent, did not induce in soil 36 as unfavorable a condition as naturally existed in soil 37. The difference in supplying power for K apparently was the dominating factor. This conclusion is not opposed to the idea that Ca, Mg, and K relations in K deficient soils are important, since both soil 36 and soil 37 contained replaceable or easily soluble Ca and Mg in such large amounts that a crucial test of the point could not be expected. We may add that these relations may be more sensitively reflected by other crops than tomato. Prune trees especially are being investigated on this supposition, with indications of positive results.

Soil 38 was then compared with soil 37, the former being a soil of relatively high supplying power for K. Five increments of K, as sulfate and as chloride, were applied to each soil. Soil 37 again gave evidence of an extremely low supplying power for K, ascribable to the very low solubility of the non-replaceable K and the minimum content of replaceable K. After increasing the amount of the latter by addition of K salt, the plant utilized replaceable K almost exclusively, in all treatments except the highest, where the value is in doubt.

The amount of K absorbed from soil 38 was significantly greater than the amount of replaceable K lost by the untreated soil or by the soil treated with the two lowest applications of K (fig. 2). The total amounts of K derived from non-replaceable form were very appreciable. At about the point representing the third application (550 p.p.m. K on basis of oven-dry soil), the lines for K withdrawn by the crop and for replaceable K lost by the soil intersect, indicating an equivalence between loss of this form of K from the soil and gain of K by the crop (tops of plant). In the two highest treatments, the amounts lost from the soil materially exceed the amounts gained by the crop. It is very difficult to explain this. Laboratory studies on this particular soil showed that it did not fix significant amounts of K in non-replaceable form when treated as in the cropping experiment.

It would seem that influences incident to cropping caused appreciable amounts of K to enter into such a form as to be non-extractable by ammonium acetate. The discrepancy cannot be explained on the basis of the K contained in the larger roots removed from the soil. Other experiments show that these values would be far too small. It is reasonable to assume that the K contained in the fine roots left in the soil would be extracted by the ammonium acetate reagent. Confidence in the results themselves is increased when it is noted that almost exactly the same curves are given by two different types of treatment, one with KCl and one with K_2SO_4. Bartholomew and Jannsen (2) advance the suggestion on the basis of one of their experiments that the growth of a crop brings about a transformation of replaceable into non-replaceable K. However it is desired now to do no more than record the facts at hand and await the results of additional experiments.

The results of analyses of the plant tissues are clear cut and warrant presentation in connection with the chemical studies on the soils. Inspection of Fig. 3 will reveal some interesting and consistent interrelations between content of K, Ca, and Mg in plant tissue; total amount of K absorbed; yields; and amounts of fertilizer applied. The two soils are in definite contrast. Certain differences of limited significance between the effects of KCl and K_2SO_4 are evident,

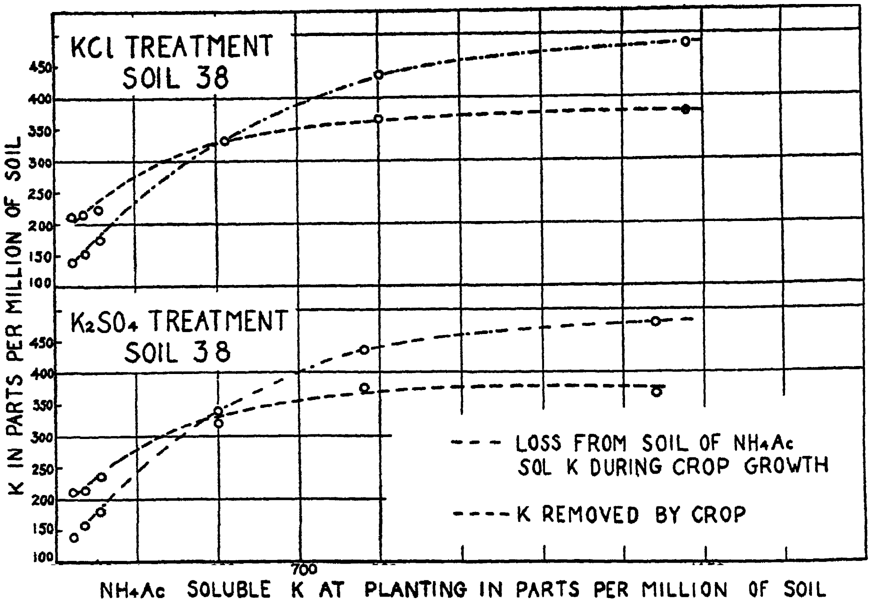

Fig. 2. Relation Between Losses of Replaceable Potassium from Soil and Gains of Potassium by Crop

Soil 38, as used in this experiment, was from a lot previously cropped. Other results of this experiment are given in figures 3 and 4. The treatments correspond approximately to a range of applications of 175 pounds to 7,000 pounds of sulfate of potassium per acre (area basis).

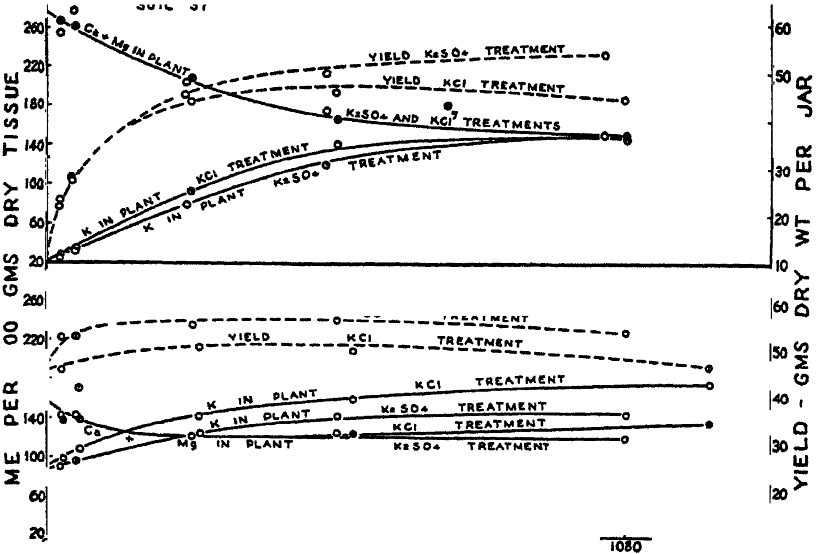

Fig. 3. Effects on Plant Composition of Different Quantities of KCl and of K_2SO_4 Added to Two Soils of Different Characteristics

especially with the higher applications. The more important aspect of this relation is the similarity of the general effects on soil and plant of applications of K in the form of the sulfate and of the chloride, in all the lower treatments, although the plants absorbed large amounts of chloride from the soil treated with the chloride. Figure 4 illustrates, for the two different soils, the relation between the amounts of K added to the soil, amounts of replaceable K, and concentrations of K in 1 to 1/2 water extracts, at the beginning of the experiment. The concentrations of water-soluble K increase at a logarithmic rate with increasing additions of K salts. As already suggested, for a given soil, the water-soluble K is evidently closely correlated with the replaceable K.

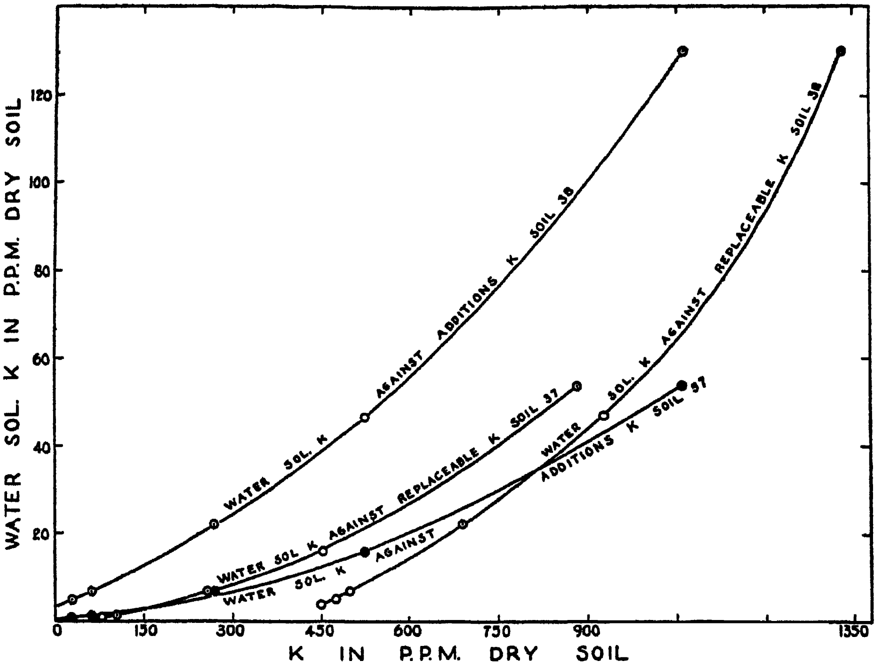

Fig. 4. Increases of Water Soluble Potassium (1 Part of Soil to ½ Part of Water) in Relation to Increments of Potassium Added to Soil and to Amounts of Replaceable Potassium Present

LONG CONTINUED CROPPING EXPERIMENTS

We shall now consider a cropping experiment which has been in progress for about 5 years. The soils already discussed were employed in this experiment, as well as a number of other soils with different characteristics. The plan of the experiment was to bring about within a few years the greatest possible withdrawal of K by plants, and to make a quantitative study of such withdrawals[5] in relation to the forms of K contained in the soil. Soils unfertilized with K were compared with fertilized soils, including in some cases two applications of K. Phosphate and nitrate were supplied at different times so that their lack would not inhibit growth. Five 5-gallon containers were employed for each treatment. The soils were kept in the greenhouse under crop most of the year, barley and tomato crops being grown in succession, as indicated in the graphs. Some drying out of the soil was allowed to occur between crops. Barley plants were grown to maturity and tomato plants to the blossoming stage.

The method of cropping just outlined makes it possible to withdraw, from a given mass of soil, amounts of K equivalent to those withdrawn under field conditions only over a period many times as long. We are able in some measure to study under control the partial exhaustion of the K supplying power of soils. It is interesting in this connection to cite a comparison between the experiments now under consideration and the tank experiments of Burd and Martin (4). In the latter, twelve barley crops absorbed from a unit mass of a certain soil only about one-half as much K as was absorbed from a similar soil by two barley crops and one tomato crop in the present experiments.

Two soils (soils 30 and 38) gave no important nor certainly significant response to K fertilization within a 3-year period. Soil 37 was deficient in K supplying power from the beginning, whereas two other soils (soils 36 and 64) developed such a deficiency in marked degree after the growth of several crops. One sandy soil (soil 53) developed a significant degree of deficiency within a limited period. Thus, the soils represented a number of well-contrasted types of behavior. Later, nine additional soils were added to the experiment and have been under observation for about 2 years.

It is now well established by observations made by many investigators that crop composition in general reflects the K supplying power of the culture medium, but apparently not many long continued systematic studies have been made on characteristically different soils under controlled conditions, with reference to this point. In this investigation, definite and almost perfectly consistent reflections of soil conditions were found in the K content of the plant tissues. The divergences in percentages of K in crops grown in different soils, or in the same soil under different treatments, were extremely large, varying, for example, between 0.15 per cent and 2.04 per cent for barley (computed on whole plant), and between 0.36 per cent and 4.05 per cent for tomato plants. Certain of the higher values imply a "luxury" consumption of large magnitude, whereas the lower values probably approximate the minimum attainable for the stages of growth concerned. The decrease in supplying power of the soils brought about by continued and intensive cropping was also reflected in extremely large decreases in the percentages of K present in successive crops. The eleventh crop (barley) had a very low percentage of K, even when grown on the soils of highest initial supplying power. Nevertheless, certain characteristic differences between soils were maintained for a long time. These enormous variations in crop composition provide an interesting basis for comparisons between chemical data on soils and on crops, but it is necessary to omit the detailed results to conserve space.

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[5] The withdrawals were primarily assignable to the K contained in the tops of the plants. Only a negligible amount of barley roots was removed from the soil. A few of the larger tomato roots were removed and an estimate of the K contained in them was made on the basis of certain analytical data. The values are too small to be of consequence, but the estimates are included in the summations.

It was observed, incidentally, that barley plants could attain lower percentages of K than could tomato plants. Although it is difficult to compare plants of such different types, especially since different stages of growth were involved, data on the plants grown under the most deficient soil condition (where practically no grain was formed by the barley plants) suggest that a physiological distinction may exist between these two types of plants, with respect to minimum percentage of K attainable. Plants of both types are evidently capable of making good growth, even when the percentage of K present in the tissues is very low, at the stages of growth studied.

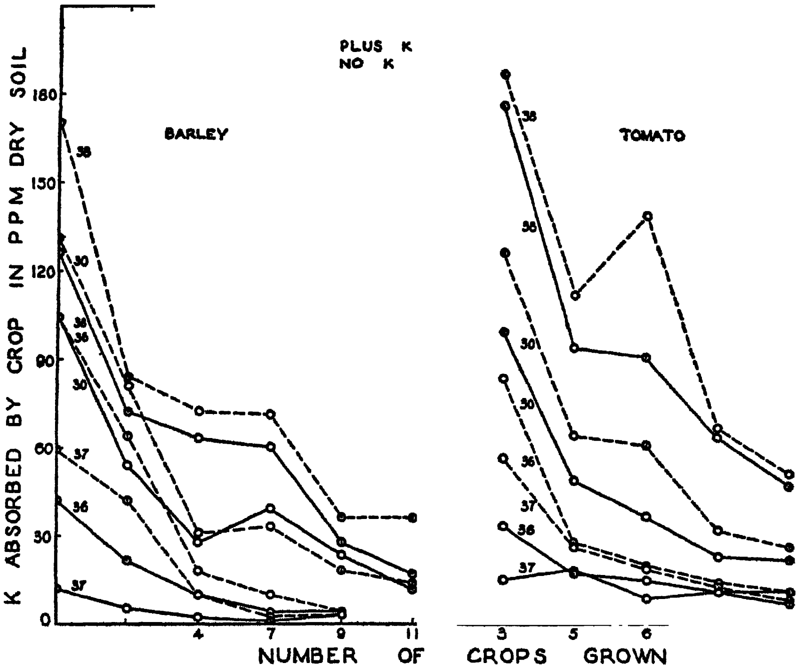

FIG. 5. EFFECTS OF CROPPING AND OF DIFFERENT SOIL CONDITIONS ON TOTAL AMOUNTS OF POTASSIUM ABSORBED BY CROPS FROM EQUAL MASSES OF SOIL

Figure 5, representing the total amounts of K removed by crops from equal weights of soil, indicates, in general, relations between the different soils similar to those pertaining to the percentage composition of the crop. The levels of withdrawal are higher for tomato than for barley, suggesting a greater absorbing power on the part of the former. This graph illustrates how radically different are the amounts of K withdrawn from soils, depending upon the nature of the soil, its treatment, and the period during which it has been cropped. Evidently extremely limited value attaches to generalizations on the rate of exhaustion of K from soils under field conditions.

RELATIONS BETWEEN POTASSIUM, CALCIUM, AND MAGNESIUM

The relations existing between the plant tissue content of K, Ca, and Mg were determined for each stage of the experiment. The ratios $\frac{\text{m.e.K}}{\text{m.e. Ca}+\text{m.e. Mg}}$ showed extreme variations (from less than 0.1 to over 3.0), consistently related to soil conditions and to the diverse physiological characteristics of tomato and barley plants. At points of decided inhibition of growth, caused by K deficiency, the ratios became very low and of similar magnitude for both plants. The relation of K to Mg apparently was as important as that of K to Ca. No consistent interrelations between Ca and Mg were discovered. Although the variations cited in the foregoing must have physiological significance, we desire to emphasize that our data imply that no specific ratio of bases, within a narrow range, is indispensable to high crop yields.

FURTHER EVIDENCE ON THE RELATION BETWEEN CONCENTRATIONS IN SOIL SOLUTIONS, CROP GROWTH, AND ABSORPTION OF POTASSIUM

Before starting the fifth crop, in the prolonged cropping experiment, one jar of soil from each of several treatments was removed, and the soil used in preparing solutions by the displacement method. These solutions reflected the conditions existing after the depletion of the soil caused by the growth of four crops, three barley crops and one tomato crop, and a subsequent period of 2 months allowed for recovery (table 3). Again, just before the eighth crop was planted, one jar of soil from each treatment was divided into two portions. One portion was used for a study of soil solution K, and the other portion was placed in a 2-gallon jar and cropped with tomato plants. Immediately after the plants were harvested, the cropped soil was examined. In other words, the effect of the growth of a single crop was ascertained using soils already depleted to a greater or lesser extent by exhaustive previous cropping (table 3A). In the supplementary experiment (table 3B), soil solution values were determined at the beginning of the third crop.

The laborious nature of the task of preparing soil solutions by the displacement method, as well as the large quantity of soil required, induced us in the latter two cases to utilize extracts made with one-half part of water to one part of soil. The study of such concentrated extracts seems to yield conclusions essentially similar to those arrived at by the study of displaced solutions. When K concentrations are very low, frequently there exists no great difference

TABLE 3

Relation between K in displaced soil solution, yield and condition of crop, and amount of K absorbed

IL AND TREATMENT	MOISTURE CONTENT AT TIME OF DISPLACING	K IN DISPLACED SOLUTION BEFORE PLANTING CROP 5	K REMOVED BY CROPS 5 AND 6	YIELDS OF CROP			SYMPTOMS OF LEAF INJURY ON TOMATO PLANTS
				Crops 5 and 6 (tomato)	Crop 4 (Barley)		
					Grain	Straw	
	per cent	*p.p.m.*	*p.p.m.**	*gm.*†	*gm.*†	*gm.*†	
30 NP	14.4	9.0	125	156	46	43	None
36 NP	19.9	3.9	36	106	36	30	Severe
36 NPK	20.0	9.8	178	196	51	54	None
37 P	24.4	2.1	28	100	5	15	Very severe
PK	24.9	8.2	149	178	46	41	None

* p.p.m. soil.
† gm. per jar.

TABLE 3A

Relation between K in 1 to ½ water extract of soil, yield and condition of crop, and amount of K absorbed

SOIL	TREATMENT	K IN 1:½ EXTRACT		K REMOVED BY CROP*	YIELDS OF CROP			SYMPTOMS OF LEAF INJURY, TOMATO PLANTS
		Before crop*	After crop		Crop,* tomato	Crop,† barley		
						Grain	Straw	
		p.p.m.‡	*p.p.m.*‡	*p.p.m.*‖	*gm.*§	*gm.*§	*gm.*§	
30	NP	5.4	0.8	23	64.3	59.6	74.7	None
	NP2K	11.0	4.0	38	66.7	61.9	75.6	None
36	NP	2.2	0.6	11	38.2	12.0	23.0	Severe
	NP2K	3.2	0.7	21	69.1	50.2	67.6	Slight
37	NP	2.0	0.5	11	43.3	0.6	5.6	Very severe
	NP2K	2.8	0.4	19	65.3	53.3	69.7	Definite
38	NP	3.2	0.3	64	94.3	60.6	73.7	None
	NP2K	6.1	1.0	102	94.4	63.2	72.8	None
53	NP	4.2	0.7	18	51.5	66.1	78.2	None
	NPK	6.6	2.9	28	70.1	66.5	77.5	None
64	NP	2.4	0.6	16	61.8	40.4	61.8	Severe
	NPK	4.5	1.3	24	71.3	64.0	71.3	None

* Eighth crop for soils 30, 36, 37, 38, and fifth crop for soils 53 and 64.
† Seventh crop for soils 30, 36, 37, 38, and fourth crop for soils 53 and 64.
‡ *p.p.m.* of extract.
‖ *p.p.m.* of soil.
. per jar.

between the concentration present in 1:½ extract and in a solution displaced at so-called optimum moisture content.

The conclusion may be drawn that a decrease in concentration of K in a soil solution to a very low point does not necessarily mean that crops cannot absorb an adequate amount of K, or even an excess amount. The question is not whether the concentration, reflecting the supplying power of the soil, is low, but whether it has dropped below critical limits. It will be recalled that plants may absorb, relative to the concentration, a larger proportion of K from solutions

TABLE 3B

Comparison of different soils with respect to K concentrations in 1 to ½ water extracts of soils, replaceable K, and crop yields

SOIL	TREATMENT	K IN 1:½ EXTRACT	REPLACEABLE K	YIELD OF CROP	
				Total dry matter	Grain
		*p.p.m.**	*p.p.m.**	*gm.*†	*gm.*†
21D	NP	4.3	48	114	44
	NPK	6.2	52	101	39
29	NP	9.6	73	121	46
	NPK	16.0	96	122	37
78	NP	2.0	23	19	2
	NPK	5.4	49	95	39
80	NP	2.4	69	158	65
	NPK	2.7	87	147	57
90	NP	1.6	69	60	14
	NPK	4.3	123	120	45
95	NP	2.9	54	153	59
	NPK	2.8	57	144	50
100	NP	0.9	56	129	52
	NPK	1.2	66	147	57
101	NP	2.4	95	132	48
	NPK	3.6	115	157	58
102	NP	1.8	14	33	5
	NPK	11.0	33	80	27

* The water-soluble K (p.p.m. of extract) and replaceable K (p.p.m. of oven-dry soil) were determined on samples taken immediately before planting, according to the procedure described in the text. The crop was grown from December, 1931 to May, 1932.

† Gm. per container, average of four.

One tomato crop was grown on these soils previous to the present experiment. The soils were then refertilized as indicated in table 4.

of low concentration than from those of high concentration. This is a mechanism of adaptation to media of low supplying power. The extent and activity of the root system must also be involved.

It is appropriate at this point to make further comparisons between concentrations of K in soil solutions and in flowing culture solutions. The various soil experiments lead to the suggestion that a magnitude of 4 to 5 p.p.m. initial concentration in a displaced solution, or 3 to 4 p.p.m. in a 1 to ½ water extract of the soil, reflect a supplying power that is adequate for barley or tomato plants under the range of general soil and environmental conditions existing during the present investigation. From solutions of initial values of 8 to 10 p.p.m., plants absorbed in some cases a large excess of K, whereas initial values of the magnitude of 2 p.p.m. or less in a displaced solution or water extract represented entirely inadequate supplying power, with perhaps one exception. Flowing culture solution experiments with tomatoes, already reported, indicated that a concentration of 5 p.p.m. at the intake was adequate even when this concentration was lowered, as a result of absorption by the crop, to 2 p.p.m. at the outlet. Concentrations of 4 p.p.m. at the intake did not give maximum yields. Concentrations of 3.7 p.p.m. falling to 0.7 p.p.m. were highly inadequate and led to marked leaf injury.

In the course of the present investigation, several additional experiments were made by the flowing solution method, using barley and buckwheat plants. Concentrations at the intake of 3 to 4 p.p.m. fairly well maintained, were found to permit the production of excellent plants, with yields only slightly, and possibly not significantly, lower than those obtained with a solution of tenfold K concentration. Experiments made at the Arkansas Experiment Station (1) led to the conclusion that a concentration of 3 to 5 p.p.m. of K was adequate for the growth of tomato plants. Parker and Pierre (19), after making experiments on corn and soybeans, estimated that adequate concentrations of K would certainly be less than 10 p.p.m. and possibly might not exceed 2 p.p.m.

On the basis of evidence now available, it appears that there is a close similarity between soil solutions and artificial culture solutions with respect to the magnitudes of the lowest concentrations of K adequate for plant growth. Unfortunately, very few results of soil experiments have been published that can be definitely compared with those now presented, so it remains to be determined whether certain soils will eventually be found to show concentrations in displaced solutions or in water extracts much lower than those we are reporting, and at the same time the soils possess adequate supplying power. In the case of phosphate, such a discrepancy between displaced soil solutions and artificial culture solutions has occasionally been found. On the other hand, it may be true of some soils that higher concentrations of K in the displaced soil solution would be required to reflect an adequate supplying power. This would depend upon the ability of the soil to maintain suitable concentrations at root surfaces, a magnitude not directly ascertainable. Some of the data furnished by

Bartholomew and Jannsen (2) suggest that the soil used in their experiments may possess a low supplying power in this sense.

If we now leave aside the lowest and also relatively high concentrations (above 10 p.p.m., for example), and consider intermediate concentrations, in comparing different soils there is no consistent interrelation between initial or final concentrations in the soil solution and amounts of K absorbed by plants. For example, at one period of the experiment, a tomato crop absorbed nearly three times as much K from soil 38 as from soil 30, yet soil 30 had an initial concentration of 5.4 p.p.m. and soil 38 only 3.2 p.p.m. (1 to ½ extracts). Other similar instances will be found in tables 3, 3A and 3B. Soil 38 appeared to maintain concentrations above critical levels, but not concentrations that would lead us to infer the very high withdrawal of K actually occurring. The supplying power of different soils is certainly no simple and general function of the soil solution concentration, as far as this concentration can be determined by means of water extracts or of displaced solutions, although, as has already been demonstrated, in a given soil fertilized with different increments of K, consistent reflections of relative supplying power may be found in the composition of the soil solution, even in soils of high fixing power (fig. 1). It is also true in the majority of cases cited that in a given soil, soil solution values reflect, over a long period of cropping, the results of previous applications of K fertilizers to the soil.

We shall not ordinarily find that the amount of K present at any one moment in the soil solution contained in the whole mass of available soil is nearly equal to the total amount absorbed by the crop during its entire growth cycle. It is true that in some western soils the amount initially found in the soil solution may be relatively adequate, but it is probable that such cases are less common than we have previously supposed. Furthermore, present evidence leads to the assumption that the high concentrations sometimes found in naturally occurring soil solutions are ordinarily confined to the very surface zones of the soil (11). In a soil recently heavily fertilized and of relatively low fixing power, soil 30 for example, the amount of K present in the soil solution at a single moment may be approximately adequate for a crop, but the condition is temporary. This is illustrated by the great drop in soil solution concentrations following the growth of a single crop. Even after a period of fallowing, the concentrations may remain far below the original concentrations [compare with Burd and Martin (4)]. Many soils have such high fixing power that, even immediately following heavy fertilization, the soil solution contains only a very low concentration of K (soils 36, 37, 38, 64, 95, etc.). These statements are based on experiments in which limited volumes of soil were available for root dispersion, but the disproportion between the amount of K present at any one time in the soil solution and the total amounts absorbed by the crop is often great enough to lead to the supposition that under field conditions, the same general conclusion holds.

ADDITIONAL EVIDENCE ON THE ABSORPTION OF POTASSIUM FROM REPLACEABLE AND NON-REPLACEABLE FORMS

Many experiments indicate that certain primary soil minerals, when finely divided, have an ability to supply K at concentrations and rates permitting satisfactory, if not maximum, growth of the plants used. Great difficulty is found in interpreting the results of such experiments in terms of soil conditions. The rate of solution and equilibrium concentration for K present in the minerals concerned will depend, in part, upon fineness of grinding and upon other conditions that may affect the surfaces of the particles. It seems essential that more studies be made directly on soils with reference to their ability to supply K to plants from replaceable or non-replaceable form. Upon this point, a marked diversity of opinion exists. Some investigators are inclined to place almost exclusive emphasis on the "adsorbed" or replaceable K, while others stress the importance of other forms. The availability of all replaceable K in certain types of soil found in Hawaii is questioned by McGeorge (12). Page and Williams (18) have discussed the complexity of the interpretation of field experiments in terms of replaceable and non-replaceable K. Recently, Oden (17) and Hissink (8) stressed the importance of replaceable K, and Gedroiz (7), of non-replaceable K. Space limitations prevent citation of many other pertinent references, but the work of Fraps (6), Wheeting (22), Breazeale (3), and Bartholomew and Jannsen (2), may also be mentioned.

Previously published results by Martin (15), on various California soils, were indicative of the importance of K in replaceable form. The data now available enable us to extend the earlier views, since the cropping procedure was far more intensive than before. Tomato plants were used, as well as barley, and new types of soil of widely varying characteristics were employed.

The total amounts of K removed from a unit weight of soil were computed for each crop, and compared with losses of replaceable K, as determined several times during the period of study. As cropping continued, decreases in amounts of replaceable K became smaller and smaller, until finally a point was attained at which the amounts recoverable by the ammonium acetate method were substantially constant, or else fluctuated in an indeterminate manner within a limited range of values and probably within the limits imposed by sampling and analytical errors (fig. 6). Consequently when the total amount of K removed from a soil by a series of crops is compared with the total amount of K lost from replaceable form it is found that the latter supplied to crops only a relatively small fraction in the case of the unfertilized soils. All, or the major portion of, the K absorbed by crops from soils like 36 and 37 may be derived from a non-replaceable form, even by the first crop. In effect, these soils have their base-exchange complex saturated with Ca and Mg, together with a small amount of Na. The extremely small value obtained for replaceable K is a maximum value, since solution from minerals of other types is also involved in the process of making the laboratory determination.

Inspection of figure 7 will quickly elucidate the relations we desire to stress. In the case of the unfertilized soils, from 55 to 100 per cent of the K absorbed by the first four crops is unbalanced by any decrease in replaceable K. The effect of fertilization is to cause a much larger proportion of the K absorbed by the crop to be balanced in this way. The loss of replaceable K from soils 30 and 38, fertilized, accounts for the entire amount withdrawn from the soil during the growth of the first four crops, but the marked decrease in amount of replaceable K brought about by cropping so altered conditions as to cause the four crops of the second series to absorb a much greater proportion of their K from non-replaceable form than did the first four crops. (For present purposes, physiological differences between tomato and barley plants may be disregarded.) Soil 37 presents an extreme case. Within the limits of interpretation of the experimental data, all the very small amount of K absorbed by any of the crops from the unfertilized soil was apparently derived from non-replaceable fractions, but an exactly opposite condition prevailed in the fertilized soil, practically all

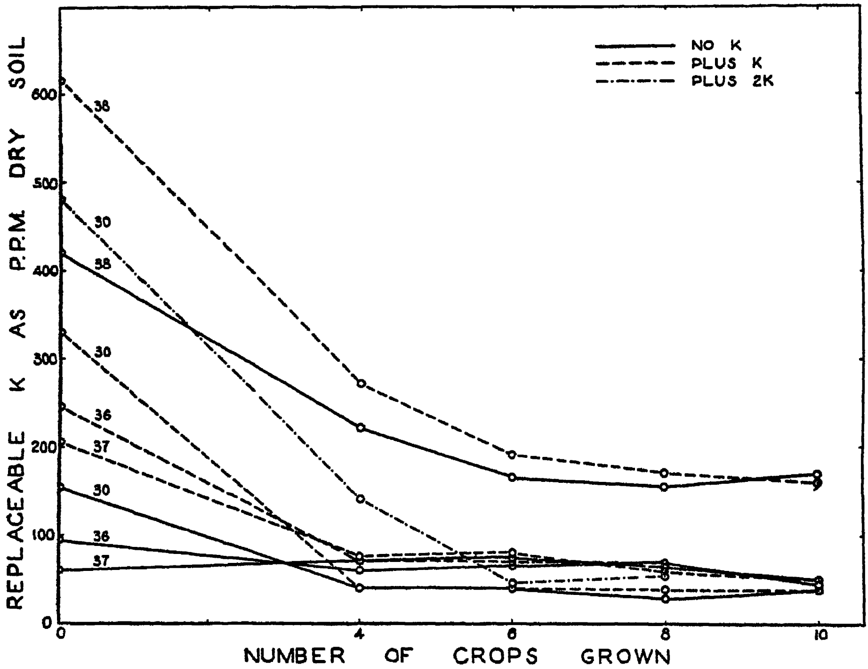

FIG. 6. DECREASE IN AMOUNTS OF REPLACEABLE POTASSIUM IN SOILS AS A RESULT OF CROPPING

Note that soil 30 attains a lower level than soil 36 or 37, although its supplying power remains much higher (see fig. 5). Crops 1, 2, 4, 7, 9, barley; 3, 5, 6, 8, 10, tomato. K_2SO_4 added at approximate rate on area basis of 2,000 pounds per acre for single treatment, and 4,000 pounds for double treatment.

the K withdrawn by the first four crops being accounted for by loss of replaceable K.

The view has sometimes been advanced that plants absorb all their K from the base-exchange complex, the K removed from this complex being continuously restored from other mineral components. Our opinion is that it is not logical to assume that plants select K from some particular type of mineral component. Rather, there is involved some sort of equilibrium, or tendency toward equilibrium, between the soil solution and all solid phase components. If, therefore, crop growth brings about no loss of replaceable K, we can only conclude that the non-replaceable K is, in effect, determining the supplying power of the soil.

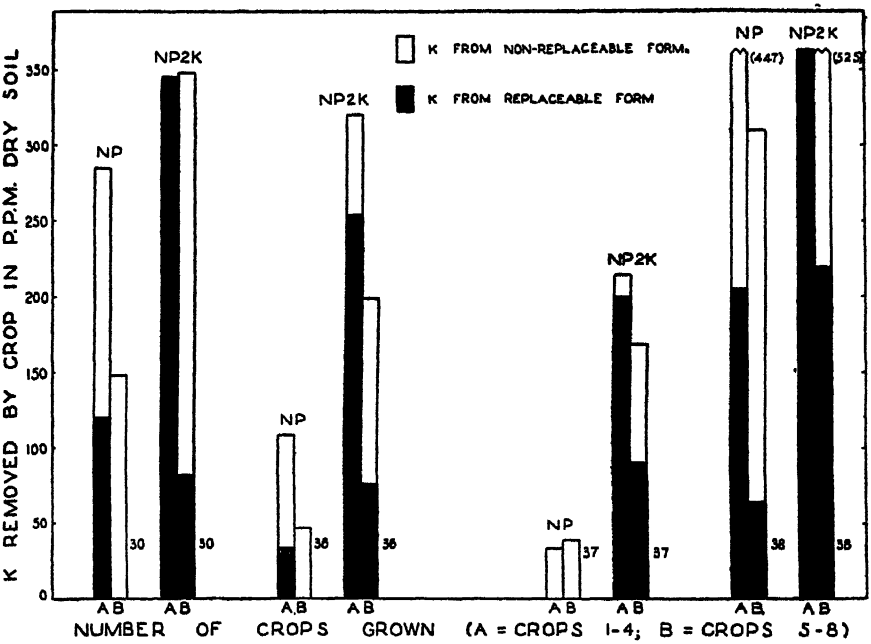

FIG. 7. RELATIONS BETWEEN POTASSIUM ABSORBED FROM REPLACEABLE AND NON-REPLACEABLE FORM

Effects of cropping, fertilization, and different characteristics of the several soils should be noted. Crops 1 to 4 were made up of three barley crops and one tomato crop; crops 5 to 8 of three tomato crops and one barley crop. In the case of the potassium fertilized soil (2 K treatments) no treatment of any kind was given until the second crop. Consequently, lack of nitrogen prevented full absorption of potassium by the first crop, especially in soil 38, but this is unimportant for the comparisons now emphasized.

The question then arises whether the supply of K derived from non-replaceable form is adequate to produce satisfactory or maximum growth of crops. Very clearly, this was not the case with soil 37 at any time, nor with soils 36, 64, 78, 90, and 102, subsequent to the growth of several crops (tables 3A, 3B, and 4, also unreported data on yields). Such a deficiency can only be ascribed to the low solubility of non-replaceable K. On the other hand, soil 30 continued to supply a sufficient amount of K for excellent growth of barley and tomato plants, for a long time after the amount of replaceable K was lowered to practically the minimum point. The concentration in the soil solution was then maintained at a very low, but not critically low, level. The replaceable K in soil 30 fell to a lower value than in soil 36 or 37, and yet soil 30 maintained a greater supplying power than the latter two soils (figs. 5, 6, and7). Soil 38 supplied to the seventh and eighth crops an adequate amount of K, but the fall in amount of replaceable K in the soil at this point was almost negligible. Another soil, a very sandy soil, still supplied an amount of K not far below the point of adequacy, after the replaceable K was reduced to only 17 p.p.m.

TABLE 4

*K absorbed from replaceable and non-replaceable form by one tomato crop from soils of different characteristics**

(Values as p.p.m. of oven-dry soil)

	SOIL 21D	SOIL 29	SOIL 78	SOIL 80	SOIL 90	SOIL 95	SOIL 100	SOIL 101	SOIL 102
NP treatment									
Loss of replaceable K from soil....	13	25	0	16	43	0	24	26	0
Total K absorbed by crop........	66	87	14	47	69	48	52	90	10
K absorbed from non-replaceable form..........................	53	62	14	31	26	48	28	64	10
Per cent of K absorbed from non-replaceable form...............	80	71	100	66	38	100	54	71	100
NPK treatment									
Loss of replaceable K from soil....	83	110	146	33	239	15	80	137	0
Total K absorbed by crop........	109	137	149	77	242	83	106	149	61
K absorbed from non-replaceable form..........................	26	27	3	44	3	68	26	12	61
Per cent of K absorbed from non-replaceable form...............	24	20	2	57	1	82	25	8	100
Per cent of K added to soil fixed in non-replaceable form...........	0	6	7	62	22	77	56	20	80

* These data pertain to second crop (tomato) grown on these soils. Refertilized after first crop (barley). The amount of K_2SO_4 added was equivalent to approximately 750 pounds per acre (area basis) for soils 21D and 102, and 1500 pounds for each of the other soils. $Ca(NO_3)_2$ and treble phosphate were also added to all soils.

Plate 1 shows at a glance how great the contrast is between barley plants grown under soil conditions, differing only in regard to the solubility of non-replaceable K as the limiting factor in growth. Two important cases are involved: (*a*) soils 30, 38, and 95, in which K fertilization had practically no influence on crop growth, even though the unfertilized soil suffered very slight, or no, lowering of replaceable K as a result of crop withdrawal; (*b*) soils 36, 37, 78, and 90, in which extremely large effects were produced by K fertilization.

The data from the supplementary experiment already referred to furnishes other evidence on the availability of non-replaceable K (table 4). In the majority of soils employed in this experiment, the amounts of K derived by the crop from non-replaceable form were greatly decreased by the addition of K to the soil, in agreement with the results already presented. However, in three soils (soils 80, 95, 102), which fixed unusually large proportions of K in non-replaceable form, an increased amount of K was derived from this latter form after fertilization of the soil. This must mean that some of the K fixed in non-replaceable form had a sufficient degree of solubility, or rate of renewal to the soil solution, to maintain a higher rate of supply to plants than could the K originally present in the soil. Yet this increased rate was distinctly inferior to that provided by soils in which fertilization increased the amount of replaceable K to a greater extent. This conclusion is based on comparison of the composition of the crops. The percentages of K present in the crops from soils 80, 95, and 102, were by no means so high as would have been found had all the K added to these soils been fixed in the replaceable state. Again we are led to the view that some forms of non-replaceable K may be adequate for the requirements of a crop, but that they do not permit a high luxury consumption. In this connection, it is to be noted that concentrations in a soil solution are always low when replaceable K is reduced to a low value (table 3B).

Although K derived from non-replaceable form may, in the case of some soils, maintain an adequate rate of supply to plants over a long period of time, it does not follow that deficiencies will not appear ultimately in every soil studied. Thus, in soils 30 and 38, definite evidence of a favorable effect of fertilization appeared in the tenth and eleventh crops. It is reasonable to suppose that not all the non-replaceable K in a soil is equally soluble and that progressive exhaustion of the more soluble forms occurs. It must be emphasized, however, that practical effects of such exhaustion might be indefinitely delayed under field conditions. In the experimental procedures, the volume of soil was limited, no part of the crop was returned to the soil, and nitrate, phosphate, moisture, and climatic conditions were highly favorable and consequently conducive to exceptionally rapid withdrawal of K. Furthermore, cropping was continued most of the year and any possible influence of fallowing was prevented or greatly limited. In view of these considerations, no surprise need be felt if response in the field to K fertilization remains negative for very long or indefinite periods in soils of the type under discussion. Soils in which the non-replaceable K has a lower order of solubility may quickly show evidence of deficiency in the field as well as in the greenhouse. Doubtless many soils found in humid regions, as well as some California soils, belong to this latter category.

The inadequacy for crop growth of certain forms of non-replaceable K naturally existing in a soil may also apply to some forms of non-replaceable K arising as a result of the addition of K to the soil. As bearing on this question, computations were made of the total amounts of K absorbed by crops from the various soils up to the period when definite evidences of deficiency first became clearly significant, and the percentages of K in the crops from the treated and untreated soils approached each other. According to the method of computation previously described, there still remained unaccounted for in the crop withdrawals from several soils a significant portion (25 per cent or more) of the K originally added to the soil. It is a reasonable assumption that the K thus unaccounted for may have entered into a very slightly soluble form in which it could not be supplied to plants at an entirely adequate rate. If this interpretation of the data is valid, then the growth of the crop itself must have caused a certain amount of K to go into a non-replaceable form in certain instances. Soil 30, when treated in the laboratory, gave full recovery of added K, even after the treated soil had stood for nearly a year, but full recovery was not obtained from 11 successive crops.

It may be true that a gradual change in the opposite direction can take place under certain circumstances, resulting in an increase in replaceable K. In one of the experiments described earlier in this paper, soils were examined following 3 months' standing in a moist condition after the crop had been harvested. A slight but consistent tendency toward increase of replaceable K was noted in several soils, but significant increases were not observed in soils having a very low solubility of non-replaceable K, as determined by crop experiments. It is presumably not important to the plant that this conversion take place when the non-replaceable K has an adequate rate of renewal to the soil solution. These aspects of the problem are receiving further quantitative study.

Although the conversion of non-replaceable forms of K into the replaceable form as a result solely of soil processes may be difficult to demonstrate, it can easily be proved that the growing of crops and the subsequent incorporation and decay of the crop residue in the soil tend to increase the amount of K extractable by ammonium acetate. For reasons obvious from this discussion, soils differ very greatly with respect to the importance of this process. An interconversion through the accumulating powers of soil microorganisms would not differ in principle from an interconversion through the agency of higher plants, but the magnitudes would be much greater in the latter case.

GENERAL DISCUSSION

Some of the main points of the discussion are summarized in a schematic form (fig. 8). In this chart, it is suggested that plants derive their supply of K directly from the solution phase of the soil, which is in dynamic equilibrium with the solid phase of the soil on the one hand, and with the plant on the other. It is clearly recognized that we have no method for obtaining samples of the physiologically effective soil solution as it may exist at root-soil particle interfaces, but that our ideas are based on the reflections of physiological conditions gained by examining displaced solutions or water extracts, which are of a composite character. The plant roots are actually dealing with many kinds of soil solutions at the various points of intimate contact between soil particles and absorbing root surfaces. The integrated effect of all these contacts can be reflected accurately only by the plant itself.

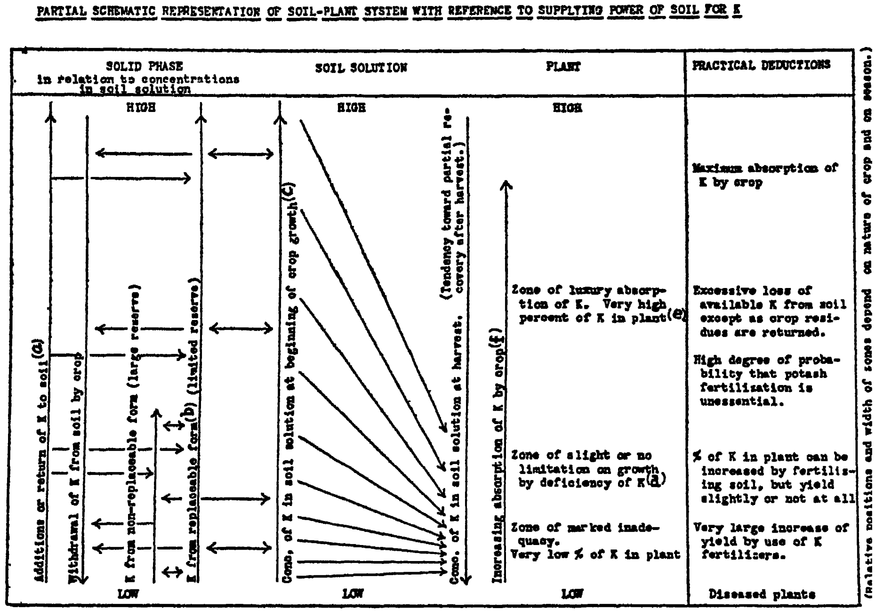
PARTIAL SCHEMATIC REPRESENTATION OF SOIL-PLANT SYSTEM WITH REFERENCE TO SUPPLYING POWER OF SOIL FOR K
SOLID PHASE
in relation to concentrations in soil solution
SOIL SOLUTION
PLANT
PRACTICAL DEDUCTIONS
HIGH
HIGH
HIGH
Additions or return of K to soil(a)
Withdrawal of K from soil by crop
K from non-replaceable form (large reserve)
K from replaceable form(b) (limited reserve)
Conc. of K in soil solution at beginning of crop growth(c)
Conc. of K in soil solution at harvest. (Tendency toward partial recovery after harvest.)
Increasing absorption of K by crop(f)
Zone of luxury absorption of K. Very high percent of K in plant(e)
Zone of slight or no limitation on growth by deficiency of K(d)
Zone of marked inadequacy.
Very low % of K in plant
Maximum absorption of K by crop
Excessive loss of available K from soil except as crop residues are returned.
High degree of probability that potash fertilization is unessential.
% of K in plant can be increased by fertilising soil, but yield slightly or not at all
Very large increase of yield by use of K fertilizers.
LOW
LOW
LOW
Diseased plants
(Relative positions and width of zones depend on nature of crop and on season.)

FIG. 8. SCHEMATIC OUTLINE ILLUSTRATING CERTAIN INTERRELATIONS BETWEEN SOIL AND CROP WITH REFERENCE TO SUPPLYING POWER FOR POTASSIUM

(a) In some soils all potassium added will enter into replaceable form; in others large amounts may become fixed in non-replaceable forms. Note that non-replaceable K absorbed by crop may build up replaceable K if crop is returned to soil.

(b) Relation of replaceable K to soil solution depends not alone on absolute value, but also on proportion of K to total exchange capacity and presumably on nature of exchange complex.

(c) A high concentration will depend on production by biological action of H ions for exchange, as well as on solid phase.

(d) Supplying power for K may be adequate even when replaceable K reaches a minimum value, provided solubility (including slow hydrolysis) of K in other forms is not too low. Zone of adequacy or inadequacy will, of course, depend on kind of crop, stage of growth, climate, etc. (Seasonal effects illustrated by Rothamsted experiments.)

(e) Any marked luxury consumption of K will usually not occur except when replaceable K is relatively high.

(f) At very high levels of absorption of K by a crop, most of the K removed from the soil is normally accounted for by loss of replaceable K. At lower levels, non-replaceable forms become of great importance. An adequate supply of K may or may not be afforded after replaceable K reaches a minimum level, depending on solubility of other forms of K. Change in quality of crop will be especially evident in same zone in which increase of yield is marked, according to data of this investigation.

It is conceived that soil solution K tends toward an equilibrium with solid phase K contained in all the constituents of the soil. The resultant equilibria determine the concentration of K in the soil solution. If the base exchange complex of the soil contains a relatively high percentage of K, as well as a relatively high absolute amount, then, in general, a high concentration will tend to be maintained in the soil solution, and a high rate of absorption by plants will take place. This may be true even when the replaceable K forms less than 5 or 10 per cent of the total of replaceable bases. When the concentration of K in the soil solution attains a sufficiently high value under the conditions described in the foregoing, the renewal of this solution, as absorption by the plant takes place, is chiefly from the exchange complex. Presumably the solution of K from other minerals is forced back by the relatively high soil solution concentration maintained by the exchange complex. However, the rapidity of absorption of K by plants is so great that marked decreases in soil solution concentrations are brought about, at least temporarily, even when K in the solid phase enters into solution readily.

As successive crops are grown without return of K to the soil, replaceable K is decreased in amount and the concentration of K in the soil solution is no longer capable of attaining a high level. K from non-exchangeable forms tends to renew the solution more easily than before, and a greater proportion of the K absorbed by the crop is derived from these non-replaceable forms. Finally, no further reduction in the replaceable K can occur and the solubility of the non-replaceable K chiefly determines concentrations in the soil solution as well as amounts held in replaceable form. When this point is reached, at which a crop can effect no further loss of replaceable K, the concentration of this element in the soil solution may or may not be maintained at an adequate physiological level, as has been indicated by the various data herein reported. If the supplying power of the soil is inadequate, recourse may be had to fertilization with K salts. K will thus be returned to the soil system and enter, wholly or in part, depending upon the soil, into dissolved and base-exchangeable form, and thus raise the levels of concentration maintained in the soil solution. Probably these levels may be raised to some slight extent by the K entering into non-replaceable form, but we have not yet met with a case of very low replaceable K (relative to total exchange capacity) and high soil solution K.

The indications are that a portion of the K fixed by some soils in a non-replaceable form will not tend to maintain physiologically effective concentrations in the soil solution much better than some of the minerals already present in the soil, and therefore this amount of K is, in effect, lost as far as practical value is concerned. Our data are not out of accord with the idea that K fertilizers should be added frequently in small amounts rather than infrequently in large amounts. A large addition may mean, not only an unnecessary loss in the crop removed from the land, but also sometimes a loss from fixation. But the fertilization of deep rooted crops is not easily accomplished by small applications when soils of high fixing power are concerned, and it is also true that some effects of heavy applications persist for a long time, as our experiments indicate. It would seem that the difficulties of fertilizing deep rooted crops, as related to differences in fixing power between different soils, has not been adequately considered in practice.

From the general point of view presented in this discussion, it is readily possible to understand why some investigators emphasize one form of K and some another. Kelley (10), in a recent discussion, warns against the tendency to make base exchange an all too embracing explanation of soil phenomena, and potassium availability seems to be a case in point.

Finally it is desirable to make a tentative statement about the rôle of organic matter as affecting availability of K. Many earlier experiments in this and other laboratories supplied quantitative evidence that the production of acids by the biological decomposition of organic matter brings about increases in soil solution concentrations, cations being dissolved as hydrogen ions are formed. The increase in cation concentration usually includes K, as well as Ca and Mg. The extent to which K concentrations can be augmented by biological activities will depend chiefly upon the replaceability by H ions of K in the various solid phases of the soil. If the replaceable form of K is present in large relative amount, then high concentrations in the soil solution will occur following a period of biological activity.

All this is reasonably clear, but a question arises to which there is not such an evident answer. Assuming that some of the K present in the solid phase of the soil is in a sufficiently soluble form, is the decomposition of organic matter essential to its solution? It may well be that the activities of root cells, including production of acids, at least carbonic acid, and the very important continuous disturbance of the equilibrium arising from the remarkable power of plants to absorb K from dilute solution, are entirely sufficient to insure an adequate supply of this element to the plant. Furthermore, according to the recent discussion of Starkey (21), bacterial activities may be very intense in root zones, organic matter for these activities being supplied by the roots themselves.

In the course of our investigation, we have treated the most deficient soils with ground sheep manure and with urea. The mere decomposition of these materials has had but little influence in making K available. The effect of the sheep manure could be assigned primarily to its actual content of K. Conceivably, over a long period of time, decomposition of organic matter may gradually increase the amount of easily soluble K in the soil, but it is uncertain how generally such decomposition is indispensable for this purpose. In nearly all experiments in which organic matter is employed, the latter contains K. It is very difficult to conclude just what effect the decomposition of organic matter *per se* has, unless the whole system is quantitatively controlled. Needless to say, these remarks have nothing to do with other important functions of organic matter.

We are well aware that the analysis just presented is only a general and tentative outline but it seems essential to have in mind some such picture of K absorption by plants as a basis for discussion and in order to unify the data now available. It goes without saying that the scheme presented will require modification, correction, and amplification as further evidence is provided.

SUMMARY

The absorption of potassium by barley and tomato plants was investigated with reference to the forms of potassium contained in the soil. Fifteen California soils of different origin and of widely different characteristics were used. In certain of the experiments, intensive cropping was carried on for over 4 years. Withdrawals of potassium from the soil were studied quantitatively. In one experiment, comparison was made between the chloride and sulphate forms of potassium. No essential difference was discovered between these salts with regard to absorption of potassium by crops or fixation of potassium by the soil.

Particular attention was given to the supplying power of the soils in relation to replaceable, non-replaceable, and soil solution potassium. Considerable light is thrown on the reasons for the diversity of views expressed by different investigators with regard to the relative physiological importance of replaceable and non-replaceable potassium.

When the amount of replaceable potassium is high, all, or the major portion of, the potassium absorbed by the crop may be balanced by loss of replaceable potassium from the soil. High replaceable potassium tends to induce "luxury" consumption of this element by the crop. As cropping continues, the proportion of potassium derived from non-replaceable form grows larger until a point is reached at which no further loss of replaceable potassium can occur, at which point the solubility of the non-replaceable form determines the supplying power of the soil. In some soils, this may be adequate for crop growth, but in other soils, the solubility of the non-replaceable form is too low and plants suffer from a deficiency of potassium.

Many of the soils investigated had the power to remove added potassium from solution almost completely. Yet by careful chemical examination, it was possible to show that concentrations in the soil solution were increased by even small applications of potassium salts. Relations between soil solution potassium and replaceable and non-replaceable potassium are discussed.

In the majority of cases, the amount of potassium present in the soil solution at any given moment was equivalent to only a very small percentage of the total amount of potassium absorbed by a crop during its growth cycle. It is now suggested that this may be a common condition even in western soils.

Comparison was made between soil solutions and artificial culture solutions with respect to concentrations of potassium below the point of adequacy for plant growth. The general magnitudes were found to be very similar. When solutions of intermediate concentration derived from different soils were compared, no consistent relation between concentration and amount of potassium

absorbed by crops was found, although in a given soil treated with varying amounts of potassium salts the relations were consistent.

The composition of the crop consistently reflects the supplying power of a soil for potassium under suitably controlled conditions. A striking interrelationship between calcium, magnesium, and potassium exists, but it is emphasized that crop growth is not limited by specific ratios of bases.

Certain soils had the power to fix a large amount of potassium in non-replaceable form immediately after fertilization of the soil. In other soils, all potassium added was recoverable by leaching the soil with ammonium acetate. The possibility is suggested that under some soil conditions, influences incident to crop growth may, in some unknown manner, cause some of the potassium added to the soil to go into non-replaceable form. In certain cases, a portion of the potassium added to the soil was not recoverable in the crop, over long periods of intensive cropping.

Attention is called to the importance of considering differences among soils in fixing power for potassium in connection with attempted fertilization of deep rooted crops.

A schematic outline is tentatively presented in an attempt to make clear certain interrelations of soil and plant with respect to potassium.

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PLATE 1

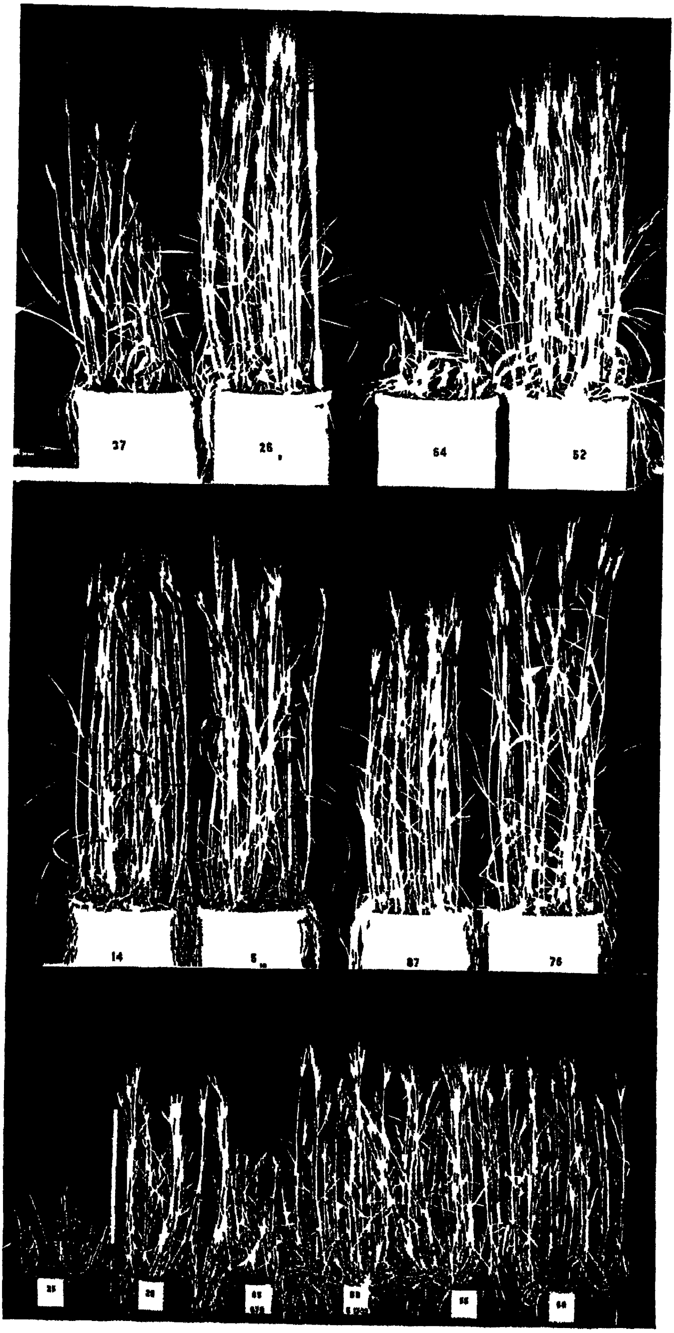
Illustrating Adequate and Inadequate Rates of Solution of Non-replaceable Potassium

All these soils had been previously cropped and the replaceable potassium reduced to very low and practically minimum values. The great differences between the soils in potassium supplying power cannot be related to replaceable potassium.

Scale of each picture is different. In each pair, right hand jar received potassium treatment.

Yields, averages of four jars, one of which is shown

UPPER PICTURE				MIDDLE PICTURE				LOWER PICTURE			
Soil	Number of jar	Total dry weight	Grain	Soil	Number of jar	Total dry weight	Grain	Soil	Number of jar	Total dry weight	Grain
		gm.	*gm.*			*gm.*	*gm.*			*gm.*	*gm.*
36	37	35	12	30	14	134	60	78	25	19	2
	26	118	50		5	138	62		28	95	39
37	64	6	1	38	87	134	61	90	85	60	14
	54	123	53		76	136	63		88	120	45
......	......	......	......	......	......	......	......	95	55	153	59
......	......	......	......	......	......	......	......		60	144	50


37
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87
76
25

THE EFFECT OF DEPTH OF PLACEMENT ON THE AVAILABILITY OF SUPERPHOSPHATE IN CALCAREOUS SOILS[1]

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The extent of penetration of phosphorus, applied as treble superphosphate, in calcareous soils is of great importance to the farmers in the irrigated areas in arid and semi-arid regions. Some soils in these irrigated areas show a marked response to the application of treble superphosphate, despite the fact that these soils are well supplied with total phosphorus. The application of 125 pounds of treble superphosphate to some soils has more than doubled the yield of certain crops, including sugar beets and alfalfa. At the same time adjacent fields have often shown no response to the same amount of phosphate fertilizer. A variation in depth of application of the fertilizer may account for the fluctuating response, if the soil is deficient in available phosphorus. Several farmers have reported that they received no increase in the crop yield the season the phosphate fertilizer was applied, but a profitable response was obtained the succeeding season.

Several investigators (1, 2, 3, 4, 5, 6) have shown that phosphorus remains largely where it is placed in the soil. In alkaline irrigated soils, which often receive less than 12 inches of precipitation and where the corrugation method of irrigation is used, the phosphate applied on the surface has little chance to penetrate. Consequently, very few plant roots may come in contact with the fertilizer and therefore no response will be shown, even though the soil may be deficient in available phosphorus. It should be stated, however, that in a few cases farmers have applied superphosphate to the surface and, by the use of the flooding method of irrigation, have obtained an immediate and profitable response.

PENETRATION OF PHOSPHATE IN A CALCAREOUS SOIL

In order to determine the extent to which the phosphorus in superphosphate penetrates the soil, an experiment was conducted by placing soil in 5-gallon jars, mixing the superphosphate with the surface 2 inches of soil, and leaching at intervals of 2 weeks. Sufficient water was added to collect approximately 1 liter of leachate at each leaching. The apparatus is shown in plate 1, figure 1. The data in table 1 show that the amount of phosphorus leached from the soil was exceedingly small. After the soil had been leached five times, soil samples were obtained from each jar at three depths, 0 to 3 inches, 3 to 6 inches, and 6 to 9 inches. The data in table 2 indicate that the phosphorus in superphosphate is not carried down below the depth to which it was placed. The treatments, except the manure, were applied by removing the surface 2 inches of soil and mixing it thoroughly with the fertilizers. The manure was mixed with the surface 4 inches. Superphosphate (45 per cent) was applied at the rate of 300 pounds per acre, manure at 10 tons per acre, sulfur at 1,000 pounds per acre, and lime at 3 tons per acre. The soil was a clay loam containing 8.26 per cent calcium carbonate and 0.18 per cent P_2O_5.

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[1] Contribution from the soils laboratory, agronomy section, Colorado Agricultural College, Fort Collins, Colorado. The writers wish to give credit to Glenn Homer, a former student, for data relative to the lysimeter experiment.

[2] Associate professor of agronomy (soils), associate agronomist, and chief agronomist, respectively.

TABLE 1

Effect of treatments on the amount of water-soluble phosphorus leached from the calcareous soil

POT NUMBER	TREATMENT	MILLIGRAMS OF PHOSPHORUS IN LEACHINGS					
		1st	2d	3d	4th	5th	Total
1	No treatment	0.028	0.013	0.051	0.052	0.032	0.176
2	Sulfur	0.030	0.012	0.041	0.051	0.032	0.166
3	Manure	0.029	0.013	0.039	0.049	0.034	0.164
4	Superphosphate	0.040	0.014	0.050	0.052	0.035	0.191
5	Superphosphate plus manure	0.061	0.015	0.054	0.053	0.035	0.218
6	Superphosphate plus lime	0.032	0.011	0.052	0.051	0.023	0.169

TABLE 2

Effect of treatments on the distribution of water-soluble phosphorus at various depths in a calcareous soil

POT NUMBER	TREATMENT	0 TO 3 INCHES		3 TO 6 INCHES		6 TO 9 INCHES	
		p.p.m. P	pH	p.p.m. P	pH	p.p.m. P	pH
1	No treatment	0.03	8.3	0.03	8.3	0.03	8.2
2	Sulfur	0.03	8.0	0.03	8.1	0.03	8.1
3	Manure	0.07	8.1	0.05	8.2	0.03	7.9
4	Superphosphate	0.80	8.2	0.03	8.3	0.03	8.2
5	Superphosphate plus manure	0.80	8.1	0.04	8.2	0.03	8.1
6	Superphosphate plus lime	0.53	8.2	0.03	8.1	0.03	8.2

After the leaching experiment was completed and the soil samples were taken at the three different depths, alfalfa was sown. The alfalfa plants 55 days old are shown in plate 1, figure 1. It is noted that superphosphate greatly stimulated the growth of alfalfa. The addition of lime with the superphosphate apparently reduced the availability of phosphorus in superphosphate. The data also indicate that sulfur did not increase the solubility of the soil phosphorus.

PLACEMENT OF SUPERPHOSPHATE

The effect of applying superphosphate at various depths to an alkaline calcareous sandy loam soil is shown in table 3 and plate 1, figures 2 and 3. Figures 2 and 3 of plate 1 show only one of the three replications of each treatment. Jars 2, 3 and 4 received 45 per cent superphosphate at the rate of 300 pounds per acre whereas jars 5 and 6 received 1,000 pounds per acre. Alfalfa seed was placed at a depth of ¾ inch. When the plants were approximately ½ inch high, they were thinned to 24 plants per jar. When they were 65 days old, 16 plants from each jar were harvested and weighed separately. The mean oven-dry weight and the probable error of the mean of the 48 plants for each treatment are given in table 3. In table 4 is given the percentage gain of the various treatments over each other, as well as the probable error of the difference.

TABLE 3

Effect of depth and rate of application of superphosphate on alfalfa

TREATMENT	MEAN WEIGHT OF 48 PLANTS
	mgm.
No treatment	31.4 ±1.1
300 pounds superphosphate at ½ inch	28.0 ±1.8
300 pounds superphosphate at 1 inch	63.5 ±3.3
300 pounds superphosphate at 4 inches	86.7 ±7.2
1,000 pounds superphosphate at 4 inches	110.7 ±2.9
1,000 pounds superphosphate at 6 inches	123.5 ±6.5

TABLE 4

Comparison of different depths and rates of application of superphosphate on alfalfa

TREATMENTS COMPARED	DIFFERENCE		DIFFERENCE DIVIDED BY P.E.
	mgm.	*per cent*	
300 pounds at ½ inch over no treatment	−3.4 ±2.1	−10.8	1.6
300 pounds at 1 inch over no treatment	32.1 ±3.4	102.2	9.4
300 pounds at 4 inches over no treatment	55.3 ±7.3	179.0	7.6
1,000 pounds at 4 inches over no treatment	79.3 ±3.1	252.5	25.5
1,000 pounds at 6 inches over no treatment	92.1 ±6.7	293.3	13.7
300 pounds at 1 inch over 300 pounds at ½ inch	35.4 ±3.7	126.8	9.6
300 pounds at 4 inches over 300 pounds at ½ inch	58.7 ±7.4	209.6	7.9
300 pounds at 4 inches over 300 pounds at 1 inch	23.2 ±7.9	36.5	2.9
1,000 pounds at 4 inches over 300 pounds at 4 inches	24.0 ±7.8	27.7	3.1
1,000 pounds at 6 inches over 1,000 pounds at 4 inches	12.8 ±7.0	11.6	1.8

These data show that there was no significant difference between the no treatment and the application of superphosphate at a depth of ½ inch, although when the phosphate fertilizer was placed at 1 inch below the surface, which was ½ inch below the seed, there was a gain of 102.2 per cent over the no treatment. The same amount of fertilizer placed at a depth of 4 inches gave a gain of 179.0 per cent over the no treatment. There was a barely significant increase in the 1,000-pound application at 4 inches over the 300-pound application at 4 inches. The increase of the 1,000-pound application at 6 inches over the 1,000-pound application at 4 inches was not significant.

SUMMARY

A study was made on the movement of phosphorus in a calcareous soil by mixing a phosphate fertilizer with the surface 2 inches of soil in 5-gallon jars. The amount of phosphorus leached through the soil was determined and was found to be exceedingly small.

The effect of depth of placement on the availability of superphosphate in a calcareous soil was determined by applying superphosphate at various depths in 5-gallon jars. The results show that the depth of applications makes a marked difference in the yield of alfalfa.

The failure to obtain a profitable response to superphosphate in some calcarious soils may be due to the improper placement of the fertilizer.

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PLATE 1

Effect of Various Treatments on the Growth of Alfalfa

Fig. 1. Effect of manure, superphosphate, lime and superphosphate, and sulfur on alfalfa.

Fig. 2. Effect of depth of application of superphosphate on alfalfa (plants 51 days old).

Fig. 3. Effect of depth of application of superphosphate on alfalfa (plants 65 days old).

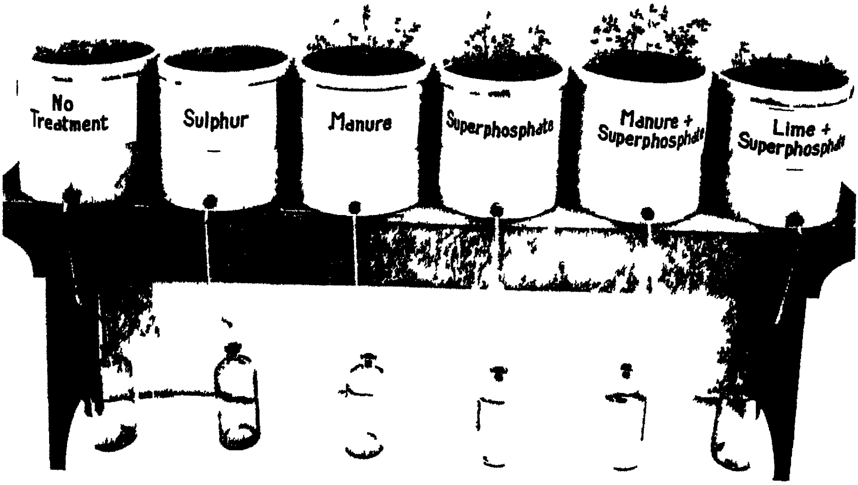

FIG. 1

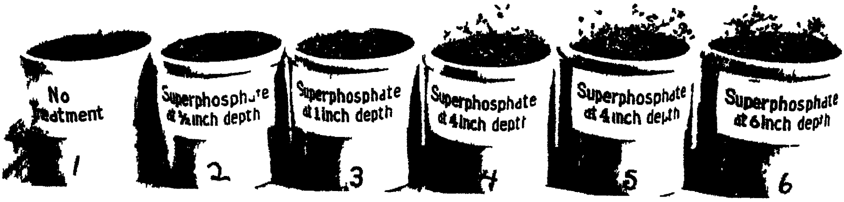
No Treatment
1
Superphosphate at ½ inch depth
2
Superphosphate at 1 inch depth
3
Superphosphate at 4 inch depth
4
Superphosphate at 4 inch depth
5
Superphosphate at 6 inch depth
6

FIG. 2

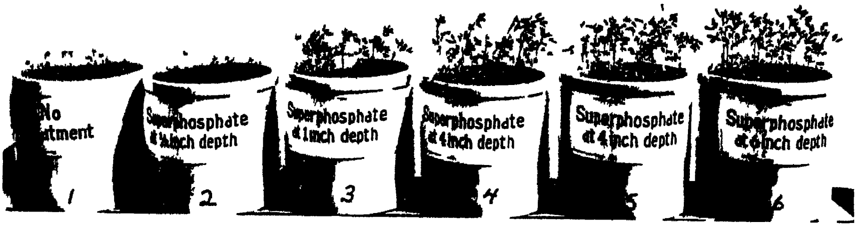
No Treatment
1
Superphosphate at ½ inch depth
2
Superphosphate at 1 inch depth
3
Superphosphate at 4 inch depth
4
Superphosphate at 4 inch depth
5
Superphosphate at 6 inch depth
6

FIG. 3

THE RELATION OF ELEVATION TO THE NITROGEN CONTENT OF GRASSLAND AND FOREST SOILS IN THE ROCKY MOUNTAINS OF COLORADO[1]

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Soil formation and development are influenced by a large number of factors, the most important of which are: climate, vegetation, parent material, and topography. More specifically, the characteristics of a soil are determined for the most part by the climatic influences, such as precipitation, temperature, evaporation, humidity, and wind velocity; by the character of the vegetative cover; by the composition and nature of the parent material from which the soil is derived; and by the topography of the surface.

Of the various soil properties which have been investigated, the total nitrogen content of the soil has attracted very general attention. The relation of climate to soil nitrogen has been thoroughly studied by Jenny (1, 2, 3, 4, 5, 6). He found that with increasing temperature, soil nitrogen decreases exponentially, and with an increasing humidity factor, soil nitrogen increases logarithmically. The term "humidity factor" is a quotient of precipitation and evaporation.

In this investigation, the influence of climate and vegetation was studied as they vary with elevation and affect the nitrogen content of the soil. An attempt was made to keep the remaining factors constant.

Soil samples were obtained at various elevations in the region between Fort Collins and the Continental Divide in the Rocky Mountains. The samples were collected at three depths, 0 to 3 inches, 3 to 6 inches, and 6 to 12 inches. Total nitrogen determinations were made on the portion of soil which passed a 1-mm. sieve.

DISCUSSION OF RESULTS

The total nitrogen content of the surface 3 inches from both grassland and forest soils is shown in tables 1 and 2. The nitrogen content of the surface 3 inches of soil is greater under forest cover than in the grassland soils. The coarse raw forest litter does not constitute a part of these samples, although at the higher elevations the surface 3 inches consisted largely of forest mull and duff. Only the portion which passed a 1-mm. sieve was analyzed and this was obviously the fairly well decomposed material. Likewise, the samples taken from the grassland were sieved through a 1-mm. sieve and the coarse roots were discarded. These data indicate that under forest cover, the nitrogen content of the surface 3 inches increases with an increase in elevation. In the grassland soils the nitrogen content, likewise increases with an increase in elevation, although above an elevation of 10,000 feet it is noted that the nitrogen content varies considerably. There is great variation in the local climate at these high elevations. The high wind velocity causes the snow to drift considerably. Some of these snow drifts remain the whole year. The samples which were obtained from peaks exposed to the wind and weather were always lower in nitrogen than the soil samples taken at the same elevation, but from

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[1] Contribution from the soils laboratory, agronomy section, Colorado Agricultural College. The senior author wishes to express his indebtedness to Dr. Hans Jenny, of the soils department, University of Missouri, for his many suggestions and criticisms during the course of this investigation.

[2] Associate professor of agronomy (soils) and graduate student, respectively.

TABLE 1

The relation of elevation to the nitrogen content of grassland soils

ELEVATION, FEET ABOVE SEA LEVEL	PER CENT OF TOTAL NITROGEN			ELEVATION, FEET ABOVE SEA LEVEL	PER CENT OF TOTAL NITROGEN		
	0 to 3 inches	3 to 6 inches	6 to 12 inches		0 to 3 inches	3 to 6 inches	6 to 12 inches
5,000	0.096	0.076	0.067	10,800	0.201	0.167	0.106
5,170	0.121	0.095	0.079	10,900	0.286	0.261	0.200
5,350	0.120	0.115	0.115	11,000	0.179	0.079	0.091
5,500	0.136	0.098	0.066	11,300	0.825	0.501	0.412
5,600	0.159	0.110	0.102	11,350	0.668	0.314	0.195
5,750	0.160	0.121	0.076	11,400	0.586	0.482	0.374
5,760	0.182	0.118	0.086	11,450	0.421	0.123	0.098
5,780	0.269	0.138	0.101	11,500	0.324	0.202	0.112
5,900	0.192	0.107	0.096	11,550	0.402	0.337	0.223
6,200	0.210	0.131	0.086	11,600	0.356	0.212	0.132
6,400	0.190	0.129	0.092	11,900	0.887	0.609	0.514
6,920	0.201	0.128	0.112	12,000	0.252	0.079	0.045
7,100	0.220	0.138	0.121	12,000	0.358	0.269	0.211
7,430	0.218	0.126	0.081	12,000	0.562	0.492	0.392
7,450	0 231	0.152	0.123	12,010	0.496	0.326	0.268
8,260	0.275	0.125	0.081	12,020	0.610	0.523	0.416
8,800	0.316	0.150	0.131	12,050	0.099	0.077	0.042
9,050	0.350	0.198	0.142	12,070	0.567	0.426	0.213
9,080	0.329	0.189	0.151	12,080	0.272	0.352	0.352
10,000	0.898	0.125	0.055	12,090	0.202	0.198	0.108
10,100	0.465	0.198	0.134	12,100	0.117	0.059	0.059
10,300	0.392	0.188	0.131	12,120	0.148	0.111	0.092
10,350	0.556	0.425	0.301	12,140	0.426	0.312	0.213
10,400	0.456	0.322	0.212	12,170	0.354	0.201	0.126
10,500	0.682	0.421	0 234	12,180	0.056	0.042	0 021
10,550	0.213	0.142	0.112	12,190	0.773	0.508	0 384
10,620	0.142	0.121	0.103	12,200	0.306	0.147	0.116

the protected areas between peaks. Vegetation is sparse on the peaks. In the region from which these samples were obtained, timber line is approximately 11,000 feet.

The data in tables 1 and 2 also show the relation of elevation to the nitrogen content of the 3 to 6 inch depth samples from both the grassland and forest soils. These data show that under grassland, the nitrogen content increases slightly up to an elevation of 10,000 feet and then it varies considerably, but under forest cover the nitrogen remains noticeably low. At the higher elevations, the nitrogen content is much higher in the grassland soils than in the forest soils at this depth.

Table 1 and 2 show the results obtained from the 6 to 12 inch depth samples. The nitrogen content of the forest soils is very low. At the higher elevations, the 6 to 12 inch samples under forest cover were ashy gray and in a few cases they were distinctly podzolized. The grassland samples from the 6 to 12 inch depth are higher in nitrogen and there is a tendency for the nitrogen to increase with elevation, although as in the surface samples, the nitrogen varies considerably at the higher elevations.

TABLE 2

The relation of elevation to the nitrogen content of the soil under forest cover

ELEVATION, FEET ABOVE SEA LEVEL	PER CENT OF TOTAL NITROGEN			ELEVATION, FEET ABOVE SEA LEVEL	PER CENT OF TOTAL NITROGEN		
	0 to 3 inches	3 to 6 inches	6 to 12 inches		0 to 3 inches	3 to 6 inches	6 to 12 inches
5,350	0.341	0.136	0.019	8,635	0.351	0.155	0.051
5,590	0.311	0.107	0.061	8,850	0.511	0.068	0.042
5,750	0.251	0.135	0.067	9,000	0.442	0.100	0.020
5,900	0.388	0.132	0.059	9,050	0.798	0.031	0.032
6,200	0.421	0.162	0.061	9,150	0.787	0.109	0.038
6,300	0.310	0.159	0.062	9,200	1.151	0.096	0.029
6,400	0.768	0.317	0.051	9,250	0.901	0.071	0.031
6,810	0.426	0.123	0.053	9,750	0.971	0.044	0.024
7,300	0.329	0.132	0.049	9,800	0.822	0.056	0.028
7,440	0.401	0.130	0.052	10,450	0.898	0.079	0.051
7,560	0.231	0.140	0.061	10,500	0.990	0.082	0.050
8,200	0.744	0.111	0.041	10,700	1.483	0.121	0.046
8,250	0.277	0.046	0.027	10,800	1.982	0.135	0.062

Table 3 gives some climatological data (7) which aid in an explanation of the results presented in this paper. It is evident that as the elevation increases, the mean annual temperature decreases and the annual precipitation increases. These data indicate that at high elevations where the temperature is low, the processes of organic matter decomposition are very slow and thus organic matter accumulates. Another factor which may contribute to organic matter accumulation is soil acidity. It was shown by the authors (8) that soil acidity increases with elevation and is greater under forest cover than in grassland soils.

It may be stated that the amount of organic matter found in the soil is a natural equilibrium between the amount produced and its decomposition by microörganisms. Thus, organic matter tends to accumulate when the processes of decomposition are slowed down and it tends to disappear when the decomposition processes are hastened (9). This investigation helps to explain the fact that in the southern states the amount of organic matter is low, a result for the most part of high temperature. In the northern states, it is higher, even though the amount of organic matter added to the soil may be the same. The beneficial effects of organic matter are well known. The farmers in the northern states can easily increase the organic matter content of their soils, whereas in the southern states, it would be rather difficult, if not impossible, to increase permanently the organic matter content.

TABLE 3

Relation of elevation to precipitation and temperature

WEATHER STATION	ELEVATION	MEAN ANNUAL TEMPERATURE	MEAN ANNUAL PRECIPITATION
	feet	*°F.*	*inches*
Fort Collins	5,000	46.5	15.06
Fry's Ranch	7,500	41.9	17.00
Moraine	7,750	40.7	16.06
Estes Park	8,956	37.4	22.36
Frances	9,300	....	26.59
Spruce Lodge	9,600	....	30.13
Silver Lake	10,500	....	38.84
Corona	11,660	26.2	46.31

SUMMARY

Soil samples were obtained from various altitudes in the Rocky Mountain region west of Fort Collins to the Continental Divide.

The surface 3 inches of soil under forest cover has a higher content of total nitrogen than the grassland soils, although the grassland soils contain more total nitrogen in the depths of 3 to 6 inches and 6 to 12 inches.

In general, the nitrogen content of the soil increases with an increase in elevation, although above 10,000 feet it varies considerably. These great fluctuations are due to variations in the amount of vegetation rather than to difference in rate of decomposition. Since in higher altitudes rainfall increases and temperature decreases, the nitrogen-altitude relation observed is in agreement with known nitrogen-climate relationships.

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SOLUBLE ALUMINUM STUDIES: III. THE RELATIONSHIP OF NITRIFICATION AND SULFUR OXIDATION TO THE ALUMINUM AND HYDROGEN-ION CONCENTRATION OF SOME VERY ACID SOILS[1]

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The rôle of biological processes in the production of acid soils has been well recognized for many years. The acids produced in the decomposition of organic matter, especially that containing nitrogen or sulfur, are relatively strong acids which have a marked effect on the pH of the soil. Likewise the production of nitrates from fertilizers containing ammonia or related compounds and the production of sulfates from free sulfur neutralize some of the soil bases and increase the acidity of the soil. These processes will proceed until the acidity of the soil becomes high enough to prevent further action by the organisms that bring about these oxidations, or until the soil colloids become desaturated.

Although the process of nitrification has been noted in acid soils for many years it was believed by some investigators to take place only around lime particles which might be present in the soil. More recently, however, it has been shown that the organisms responsible for nitrification are able to oxidize ammonium compounds to nitrates even in an acid solution. The pH value which has been found to inhibit nitrification has varied, depending on the soils used and the methods of study. Gerretson (4) reported that nitrification in solution was not stopped until the pH reached 3.9. Using fresh soil, Waksman (9) found that nitrification of ammonium sulfate stopped when the pH of the soil reached 4.8 to 4.4. In recent studies with soil, Olsen (6) found that nitrification could take place at pH 3.65 but that the process was much slower than at higher pH values obtained by the addition of lime. Inasmuch as this latter investigator included in his work a review of the more recent literature showing the relationship between pH and nitrification, no further review need be given here.

In view of the difference in the limiting pH value for nitrification it would seem that some other factors might be important in limiting this process. Organisms from different soils have been found to vary in their tolerance to acidity. The salt content of the soil is also an important factor, since soluble salts have been found by many investigators to influence nitrification. Moreover, both acidity and salt content in the soil have been shown to be primary factors in determining the concentration of soluble aluminum in the soil solution (7). The effect of this soluble aluminum upon nitrification, however, has been given very little attention. Denison (3) found that the addition of soluble aluminum salts to the soil depressed nitrification but he did not determine the amount of aluminum which remained in solution. The influence which he noted might also be explained by the increased acidity which was undoubtedly brought about by these adidtions. The effect of nitrification in bringing aluminum into solution has likewise been largely a matter of conjecture. Abbott, Conner, and Smalley (1) found that nitrification took place in soils which they believed had insufficient calcium and magnesium to neutralize the acids produced and, as a result of the analysis of the water extract of the soils, suggested that aluminum might serve as the base. Mirasol (5) also stated that the production of aluminum nitrate in the soil as a result of nitrification was possible but did not give any experimental evidence to support his view. On the other hand Denison (3) stated that it was doubtful whether aluminum could replace calcium as an available base in nitrification. It would appear likely, however, that since nitrification does increase the acidity of the soil it might be directly or indirectly responsible for the solution of some aluminum, provided the pH of the soil is sufficiently low for aluminum to be dissolved. The increase in soluble salt content as a result of nitrification would also tend to increase the aluminum in the soil solution (7).

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Sulfur oxidation was found to increase the soluble aluminum in the soils studied by Ames and Boltz (2) and by Denison (3). Inasmuch as sulfur oxidation reduces the pH of the soil and takes place at very low pH values it seems probable that this biological process might, under certain conditions, be an agent in the solution of aluminum compounds. It would also seem logical that the presence of soluble aluminum would have little effect on sulfur oxidation, since the organisms responsible for the process are active in soils which are sufficiently acid to have large amounts of aluminum in solution.

Recent advances in analytical procedure have made possible more accurate determinations of the amount of soluble aluminum present in the soil. Therefore a study of the relationship between aluminum, nitrification, and sulfur oxidation is made possible. This study was made with the following objectives:

To determine the rôle of nitrification and sulfur oxidation in the production of soluble aluminum.

To determine the effect of soluble aluminum on nitrification and sulfur oxidation.

To determine the relationship between the pH of soils and nitrification and sulfur oxidation.

To study the relationship between calcium and nitrate nitrogen in the soil solution of very acid soils.

EXPERIMENTAL

Eighteen very acid soils which had been used for the determination of soluble aluminum in the soil solution were used for this study. The description of these soils has been given in a previous paper (7). Some of these soil samples were treated with ammonium sulfate at the rate of 300 pounds per acre and others with sulfur at the rate of 125 pounds per acre, as shown in table 1. In all of the samples a part of the soil remained untreated. These samples were placed in 2-gallon pots and incubated at room temperature for about 4 months at approximately optimum moisture content. At the end of this period the soil solutions were obtained by displacement and analyzed. The analytical methods were the same as previously reported (7) except that Snyder's (8) method for sulfates was used with slight modification. The comparative analysis of the soils and soil solutions at the beginning and end of the incubation period are given in tables 1 and 2.

Table 1 shows that there have been marked changes in the soil solutions during the incubation period. The hydrogen-ion concentration and nitrate content have increased in all of the soils; the salt concentration has increased in all soils except the untreated soil 575; the soluble aluminum concentration has increased in all except untreated soils 548 and 574; and the sulfate content has increased in most of the soils. The pH values obtained for the soil solutions

TABLE 1

The relationship between certain biological processes and the pH, aluminum concentration, and specific resistance of the soil solution

SOIL NUMBER*	SOIL TYPE	pH		SPECIFIC RESISTANCE 25°C.		Al		NO_3-N		SO_4	
		1†	2	1	2	1	2	1	2	1	2
						p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
546 A	Elkton silt loam	4.60	3.73	2,584	445	0.54	12.75	6.8	210.0	tr.	37
546 B		4.60	3.75	2,584	350	0.54	26.33	6.8	247.0	tr.	364
547 A	Keyport loam	4.80	4.06	2,281	784	1.03	9.00	2.0	104.0	25	25
547 B		4.80	4.43	2,281	540	1.03	3.25	2.0	37.7	25	470
547 S		4.80	4.12	2,281	672	1.03	18.40	2.0	12.8	25	505
548 A	Portsmouth loam	4.25	3.77	1,459	717	3.88	3.62	tr.	5.2	238	441
548 B		4.25	3.68	1,459	385	3.88	20.75	tr.	5.4	238	1,066
548 S		4.25	3.33	1,459	348	3.88	55.00	tr.	4.9	238	1,559
554 A	Dekalb loam	4.60	3.73	1,901	358	3.05	60.40	21.5	327.0	81	80
561 A	Monongahela fine sandy loam	4.60	4.04	1,122	433	1.31	5.78	57.5	240.0	tr.	27
561 B		4.60	3.70	1,122	218	1.31	39.00	57.5	495.0	tr.	312
561 L A		4.35	4.20	1,010	613	0.81	3.17	31.0	135.0	tr.	14
561 L B		4.35	3.81	1,010	222	0.81	33.50	31.0	440.0	tr.	449
567 A	Canfield silt loam	4.16	3.76	495	281	5.75	10.25	212.5	413.0	tr.	50
567 L A		4.20	3.88	1,134	334	0.88	8.50	65.0	256.0	tr.	26
569 A	Brooke silt loam	4.34	3.75	261	189	5.50	5.65	34.7	283.0	149	229
569 B		4.34	3.78	261	155	5.50	13.00	34.7	358.0	149	701
569 L A		4.50	3.90	1,344	295	tr.	5.83	16.0	345.0	tr.	54
569 L B		4.50	3.87	1,344	196	tr.	16.75	16.0	523.0	tr.	366
571 A	Miami silt loam	4.45	3.98	148	109	12.25	41.50	387.5	818.0	2,067	1,647
571 L A		4.25	4.23	671	534	3.83	8.25	39.0	124.0	344	404
574 A	Lenore fine sandy loam	4.04	3.86	974	825	11.82	5.70	6.2	19.9	tr.	109
574 B		4.04	3.85	974	514	11.82	22.00	6.2	18.4	tr.	505
575 A	Onslow silt loam	4.00	3.77	399	518	1.50	6.06	28.0	160.0	115	66
575 B		4.00	3.72	399	353	1.50	14.87	28.0	177.5	115	520
580 A	Canfield silt loam	4.05	3.56	844	437	11.63	32.75	100.2	249.5	29	85
580 B		4.05	3.50	844	410	11.63	42.00	100.2	238.0	29	230
583 A	Dekalb silt loam	3.98	3.53	862	495	22.63	58.00	14.9	170.0	155	212
583 S		3.98	3.52	862	430	22.63	83.00	14.9	107.0	155	807
584 A	Dekalb silt loam	3.91	3.58	898	290	27.25	110.70	13.9	265.0	154	650
584 B		3.91	3.62	898	139	27.25	190.00	13.9	185.0	154	3,551
584 L A		3.90	3.73	1,768	726	3.00	22.63	12.0	112.0	tr.	64
584 L B		3.90	3.59	1,768	392	3.00	70.50	12.0	208.0	tr.	376

TABLE 1—*Concluded*

SOIL NUMBER*	SOIL TYPE	pH		SPECIFIC RESISTANCE 25°C.		Al		NO$_3$-N		SO$_4$	
		1†	2	1	2	1	2	1	2	1	2
						p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
587 A	Holston silt loam	4.95	3.87	2,268	584	tr.	3.82	9.7	170.0	tr.	7
587 B		4.95	3.74	2,268	309	tr.	22.75	9.7	367.0	tr.	61
588 A	Dekalb silt loam	4.52	3.98	486	288	3.25	4.90	50.3	276.0	21	165
588 B		4.52	3.81	486	180	3.25	17.10	50.3	538.0	21	553
590 A	Elk fine sandy loam	4.51	3.98	3,429	714	0.56	4.95	2.7	135.0	tr.	21
591 A	Westmoreland silt loam	4.64	3.83	1,714	360	0.40	8.87	2.7	290.0	tr.	30
592 A	Dekalb silt loam	4.55	4.14	2,209	491	0.88	3.17	tr.	159.0	tr.	11
592 B		4 55	3 87	2,209	302	0 88	10.87	tr.	314.0	tr.	96

* The letters following the soil number refer to the treatment. Those marked *A* had no treatment, *B* had ammonium sulfate at the rate of 300 pounds per acre, *S* had sulfur at the rate of 125 pounds per acre, and *L* were leached before the first displacement.

† The numeral *1* refers to the first displacement made on soils brought directly from the field; the numeral *2* refers to the second displacement made after about 4 months' incubation at optimum moisture content.

after incubation are unusually low. This may be partly explained by the increased salt concentration, since an increase in salt concentration or decrease in specific resistance increases the hydrogen-ion concentration of soils. However, inasmuch as the nitrates and sulfates produced are probably largely responsible for the increased salt concentration, their production is probably the principal cause of the lowered pH.

The amounts of aluminum in the soil solution are very high but since, as has been previously reported (7), the aluminum in solution is correlated with hydrogen-ion and salt concentrations, high amounts of aluminum would be expected in these soils. It is extremely doubtful whether such concentrations would exist in field soils inasmuch as the leaching of the soil following rains would undoubtedly keep the salt concentration at a much lower level and this would in turn tend to increase the pH (7).

A fairly high accumulation of nitrates in the soil solution is also evident from the data in table 1. This is surprising in view of the high hydrogen-ion concentration, and the high aluminum and salt content. Apparently nitrification has proceeded in all of the soils studied, the amount varying in different soils and under different treatments. As a rule, more nitrates were produced in the soils receiving applications of ammonium sulfate, in spite of the increased acidity which often resulted. Oxidation of the sulfur in the soil and of added sulfur also occurred in most of the soils. This would be expected inasmuch as some of the organisms responsible are very tolerant to acidity.

TABLE 2

The pH of the soil extract and the concentration of nitrate nitrogen, sulfates, and calcium expressed on the basis of dry soil

SOIL NUMBER	pH SOIL EXTRACT		MOISTURE CONTENT AT TIME OF DISPLACEMENT		NO_3-N		SO_4		Ca	
	1*	2	1	2	1	2	1	2	1	2
			per cent	*per cent*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*		*p.p.m.*
546 A	4.80	4.24	....	15.7	....	33.0	tr.	5.8	....	32.7
546 B	4.80	4.12	....	14.5	....	35.8	tr.	52.8	....	38.4
547 A	4.70	4.57	13.7	12.4	0.3	12.9	3.4	3.1	....	7.9
547 B	4.70	4.66	13.7	13.5	0.3	5.1	3.4	63.5	....	4.5
547 S	4.70	4.26	13.7	12.6	0.3	1.6	3.4	63.6	....	6.7
548 A	4.23	3.94	20.9	17.6	tr.	0.9	49.7	77.6	....	9.7
548 B	4.23	3.93	20.9	20.1	tr.	1.1	49.7	214.3	....	21.5
548 S	4.23	3.74	20.9	17.6	tr.	0.9	49.7	274.4	....	33.6
554 A	4.53	3.92	17.0	15.5	3.7	50.7	13.8	12.4	....	32.9
561 A	4.83	4.55	11.6	14.4	6.7	34.6	tr.	3.9	....	45.1
561 B	4.83	4.16	11.6	13.3	6.7	65.8	tr.	41.5	....	82.9
561 L A	4.97	4.55	14.4	14.0	4.5	18.9	tr.	2.0	....	25.8
561 L B	4.97	4.21	14.4	13.5	4.5	59.4	tr.	60.6	....	71.8
567 A	4.40	4.27	19.7	19.1	41.9	78.9	tr.	9.6	....	80.6
567 L A	4.65	4.29	19.7	17.3	12.8	44.3	tr.	4.5	....	56.4
569 A	4.50	4.37	16.4	21.3	5.7	60.3	24.4	48.8	....	143.6
569 B	4.50	4.34	16.4	21.0	5.7	75.2	24.4	147.2	....	173.5
569 L A	5.02	4.50	21.0	20.2	3.4	69.7	tr.	10.9	....	90.1
569 L B	5.02	4.13	21.0	21.2	3.4	110.9	tr.	77.6	....	146.5
571 A	4.50	4.24	19.6	18.0	76.0	147.1	405.1	296.5	....	178.2
571 L A	4.37	4.37	18.4	20.8	7.2	25.8	63.3	84.0	....	44.9
574 A	4.35	4.38	....	20.1	....	4.0	tr.	21.9	....	6.6
574 B	4.35	4.12	....	....	....	....	tr.	.....	....	.....
575 A	4.25	4.14	18.8	21.8	5.3	34.9	21.6	14.4	....	35.1
575 B	4.25	4.09	18.8	20.0	5.3	35.5	21.6	104.0	....	43.2
580 A	4.03	3.78	25.2	33.1	25.3	82.6	7.3	28.1	....	71.5
580 B	4.03	3.74	25.2	35.4	25.3	84.3	7.3	81.4	....	79.3
583 A	4.00	3.88	22.2	22.0	3.3	37.4	34.4	46.6	....	20.7
583 S	4.00	3.79	22.2	21.1	3.3	22.6	34.4	170.3	....	21.1
584 A	4.00	3.85	20.6	17.0	2.9	45.1	31.7	110.5	....	23.0
584 B	4.00	3.71	20.6	23.7	2.9	43.8	31.7	841.6	....	35.6
584 L A	4.25	3.94	16.2	16.5	1.9	18.5	tr.	10.6	....	9.2
584 L B	4.25	3.91	16.2	16.1	1.9	33.5	tr.	60.5	....	13.0
587 A	4.85	4.37	19.8	....	1.9	....	tr.	.....	....	.....
587 B	4.85	4.19	19.8	22.2	1.9	81.5	tr.	13.5	....	83.9
588 A	4.60	4.28	17.1	16.6	8.6	45.8	3.6	27.4	....	87.6
588 B	4.60	4.22	17.1	17.9	8.6	96.3	3.6	99.0	....	162.0
590 A	4.60	4.53	19.6	18.9	0.5	25.5	tr.	4.0	....	29.3
591 A	4.68	4.27	26.2	29.1	0.7	84.4	tr.	8.7	....	89.3
592 A	4.45	4.52	24.0	21.6	tr.	34.3	tr.	2.4	....	43.8
592 B	4.45	4.33	24.0	22.0	tr.	69.1	tr.	21.1	....	77.0

* The numeral *1* refers to the first displacement; the numeral *2* to the second displacement.

The pH of the soil extract is given in table 2. Although the hydrogen-ion concentration of the soil has increased in almost all the soils, the change is usually not as great as that in the soil solution. It will also be noted that the pH of the soil extract is usually considerably higher than that of the soil solution. This is probably due to the lower salt concentration in the 1:5 water extract used for this determination. For this reason it would seem probable that the pH of the soil solution is more nearly the correct value.

The moisture contents at the time of displacement and the nitrate nitrogen, sulfate, and calcium contents on the basis of dry soil are also given in table 2. These latter values calculated from the concentrations present in the soil solution allow more direct comparisons of the amounts found. However, the moisture contents varied so little that the relative values for sulfates only were changed and these in but a few cases.

Calcium was not determined on the first solutions obtained; therefore no comparisons of increase in calcium can be given. However, it will be noted that the concentrations of calcium were very low after incubation in soils 547, 574, and 584L, and high in soils 569, 571, and 588. It is also evident that these same soils are low and high, respectively, in nitrate nitrogen. This relationship will be discussed in detail later.

DISCUSSION

The data presented show that both nitrification and sulfur oxidation are important in bringing aluminum into solution in very acid soils. The rôle of nitrification may be seen especially in soils 547A, 554A, 561A, 561LA, 567LA, 571A, 575A, 590A, and 592A. These all show a marked increase in soluble aluminum in the soil which was probably the result of nitrification, since very little sulfur oxidation took place in any of these soils. In addition to the aforementioned soils most of those treated with ammonium sulfate also showed considerable nitrification and an increase in soluble aluminum without any apparent oxidation of sulfur, since the amount of sulfate found was no more than the 109 p.p.m. added in the ammonium sulfate. It is realized that certain biological agencies other than nitrification may be responsible in bringing some of the aluminum into solution, but undoubtedly the strong nitric acid produced in nitrification has had an important part in dissolving some of the soil aluminum.

The rôle of sulfur oxidation in the solution of aluminum in the absence of nitrification may be seen in soils 547S and 548S. In these soils there has been a noticeable oxidation of sulfur, which has increased the aluminum content of the soil solution. In some of the other samples there has also been some sulfur oxidation, which has undoubtedly contributed to the solution of aluminum.

Soluble aluminum does not appear to exert a marked influence on nitrification. Although the aluminum content of the soil solution at the beginning of the experiment varied from a trace to 27.25 p.p.m., there was a significant nitrification in all of the soils. Moreover, the amounts of nitrate produced in the soils having the highest amount of aluminum were 42.2 and 40.9 p.p.m. These amounts are much higher than the increases in many of the soils which contained smaller amounts of aluminum. The increase in the aluminum content from 27.25 to 190 p.p.m. in soil 584B was undoubtedly a gradual process, more aluminum being dissolved as the soil solution became more acid and higher in salt concentration. Likewise the nitrification probably was a gradual process which took place during a considerable part of the incubation period. It seems very probable then, that nitrification actually took place in the presence of a considerably higher concentration of aluminum than the 27.25 p.p.m. present in the soil solution at the beginning of the incubation period.

The three soils treated with sulfur showed a marked sulfur oxidation in the presence of 1.03 to 22.63 p.p.m. aluminum. Certain of the other soils also showed some oxidation of sulfur, the most noticeable being soil 584, which had the highest concentration of aluminum at the beginning of the experiment. Apparently sulfur oxidation was not prevented in this soil by the 27.25 p.p.m. of aluminum present.

The rôle of nitrification and sulfur oxidation in increasing the hydrogen-ion concentration of soils is so well known as to need little comment. Undoubtedly these processes have been active in reducing the pH of each of the soils studied. The presence and activity of nitrifying organisms in all of the soils studied are evident from the tables. Even soil 584LB, which had an initial of 3.90, supported an active nitrifying flora. Whether the organisms were active throughout the incubation period until the pH was reduced to 3.59 in the ammonium sulfate treated soil is a matter of conjecture. However, it seems probable that their activity continued below the original pH value of 3.90, especially since sulfur oxidation did not occur in this soil. Nitric and sulfuric acids are probably the strongest acids produced during the decomposition of organic matter and would be expected to exert the greatest influence on the hydrogen-ion concentration of the soil. The process of nitrification would therefore be the principal agent in the example cited in the foregoing and probably occurred until the soil was near the final pH value of 3.59. In soil 580B the same reasoning would attribute to nitrification the reduction in pH of the soil solution from 4.05 to 3.50 inasmuch as the sulfate content on the basis of dry soil was less than the 109 p.p.m. added in the ammonium sulfate. Nitrification then in this soil probably took place until the pH of the soil was almost 3.50.

Sulfur oxidation was also effective in reducing the pH of the soil solution, the most noticeable effect being in soil 548S, which had a final pH of 3.33. Sulfur oxidation was also very marked in soils 583 and 584. These latter soils are somewhat heavier in texture, and the change in pH was not as marked.

A general relationship between the calcium and nitrate nitrogen content has been suggested previously. This is more evident in figure 1, in which the nitrate nitrogen content was plotted against the calcium content of the soil solution. By statistical analysis the coefficient of correlation was found to be 0.858. The corresponding coefficient of determination was 0.737, which shows that over 73 per cent of the variation in nitrate nitrogen was associated with the calcium content of the soil solution. The regression equation was found to be $y = 11.68 + 0.6007x$. Inasmuch as the relationship in $Ca(NO_3)_2$ is $y = 0.7x$, it is evident that some nitrification took place in the absence of sufficient calcium to neutralize the acid produced. However, there were undoubtedly other bases present, such as magnesium and potassium, which could neutralize the nitric acid produced. It would appear, therefore, that in these soils nitrification has taken place only very slowly in the absence of strong bases and that aluminum cannot serve as a base for nitrification, as has been suggested by some investigators (1, 5). This does not, however, nullify the influence of nitrification in bringing aluminum into solution, for by the neutralization of the bases the soil becomes more acid, thus allowing more aluminum to be dissolved.

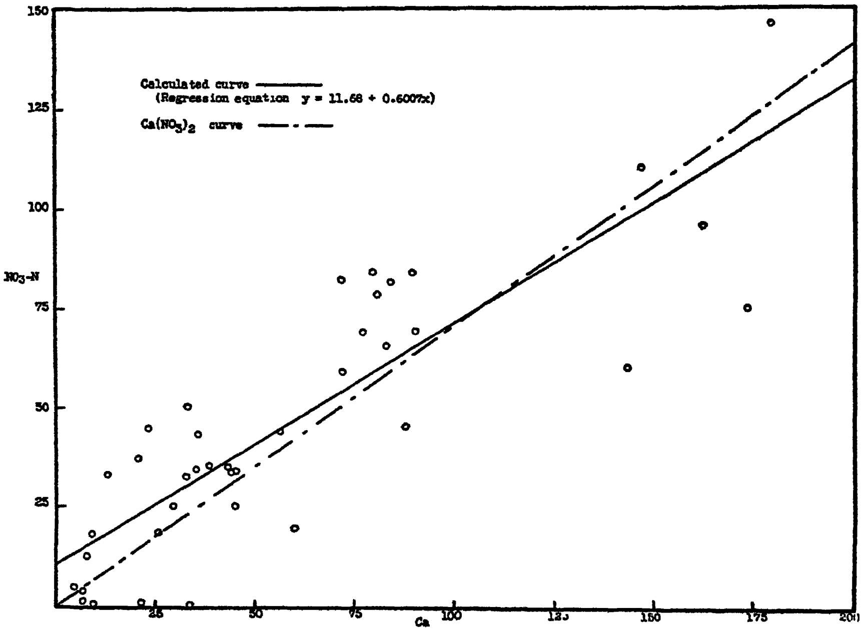

FIG. 1. THE RELATIONSHIP BETWEEN CALCIUM AND NITRATE NITROGEN IN THE SOIL SOLUTION

SUMMARY AND CONCLUSIONS

Experimental evidence showing the relationship of nitrification and sulfur oxidation to the hydrogen-ion concentration and aluminum content of the soil solution of 18 very acid soils is presented. The data may be summarized briefly as follows:

Nitrification and sulfur oxidation were effective in bringing aluminum into solution in soils, the displaced solution of which varied from pH 4.95 to pH 3.90.

Nitrification was not prevented by concentrations of aluminum present in the soil solution of any of the soils studied. Nitrification took place in one soil which had an initial concentration of 27.25 p.p.m. aluminum and a final concentration of 190.0 p.p.m. aluminum.

Sulfur oxidation was not prevented by concentrations of aluminum in the soil solution up to 27.25 p.p.m., the highest amount present in any of the soils at the beginning of the experiment.

Nitrification was found to take place in one soil at pH 3.90 and was also probably the principal agent in reducing the pH of another soil to 3.50.

Biological activities in the soil reduced the pH of the soil solution from several of the soils below 3.6, the lowest values being 3.33 in a soil treated with sulfur, and 3.50 in a soil treated with ammonium sulfate.

A marked correlation ($r = 0.858$) was found to exist between the calcium and nitrate nitrogen contents of the soil solutions studied.

These results indicate that in soils the process of nitrification is limited principally by the amount of calcium available as well as by the acidity of the soil. Further studies are in progress to determine the limiting pH for nitrification on these soils.

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CONTRIBUTION TO OUR KNOWLEDGE OF THE CHEMICAL NATURE AND ORIGIN OF HUMUS: III. THE BASE-EXCHANGE CAPACITY OF "SYNTHESIZED HUMUS" (LIGNO-PROTEIN) AND OF "NATURAL HUMUS" COMPLEXES[1]

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The function of humus in the base-binding properties of the soil has attracted in recent years considerable attention from soil investigators. This is due to the rôle of humus in removing the sesquioxides of iron and aluminum from the surface layers of podzol soils, to the reactivity of humus with the inorganic soil colloids, and to the part that humus plays in the characteristic and important phenomenon of the soil known as the base-exchange capacity. By determining the base-exchange capacity of the unmodified soil, then destroying the organic matter either by burning off or by treatment with certain chemical reagents, such as H_2O_2, and again measuring the base exchange of the residual inorganic portion of the soil, one is able to demonstrate that humus plays an important part in this process; the difference between the two determinations is usually considered to be due to the humus fraction of the soil colloids.

In certain investigations, a part of the soil humus was removed by treatment with an alkali solution. On acidification of the solution, a precipitate is formed; this is removed by filtration and washed with water. This washed precipitate, usually referred to as "humic acid" or "humus," has been frequently used for studies of the combination of humus with bases (20). It has commonly been assumed that this humus fraction, even when isolated only from highmoor peat, is characteristic of the humus of both organic and mineral soils. This assumption is hardly justified, when one considers the difference in the chemical composition of the plants and in the processes of decomposition. Further, since this fraction represents only a part of the soil humus, the results obtained would have to be considerably modified before they could be interpreted in terms of the soil organic matter as a whole. It is quite probable that the base-absorbing or base-exchange complex of the soil largely consists of both clay and humus fractions in a definite combination (5, 21).

The nature of interaction between neutral salts and soil humus, the absorption of bases, and the liberation of acids have attracted considerable attention in the past. A whole literature has accumulated on this subject. An attempt has even been made to interpret the chemical nature of soil humus on the basis of this process.

Eichhorn (6) was among the first to demonstrate that when peat and humates are allowed to interact with salts, an exchange of bases will take place. König (13) concluded that humus is able to bind mechanically the alkali from alkali solutions. The higher the ash content of the peat the less is the amount of alkali absorbed, this absorption being highest in the case of sphagnum peat and lowest in lowmoor peat. König explained the difference in the behavior of the two types of peat by the greater decomposition of the latter. This led him to the generalization that the more decomposed a peat is the lower will be its absorbing capacity for bases. This is totally incorrect, since these two types of peat represent totally different chemical aggregates, aside from their base content.

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The absorption of bases from salts by humus complexes was considered by most investigators to be a chemical process. In many instances, observations made on the adsorption processes by coal or by artificial humus prepared from sugar were applied to explain the function of humus in the soil. Here again, conclusions were based upon insufficient evidence. The general conception of this process can best be expressed by the idea of Berthelot and André, namely,

$$H_2\text{-humate} + CaCl_2 = Ca\text{-humate} + 2HCl$$

This reaction was believed to explain the fact that when humus, poor in bases, is allowed to interact with salts acids are liberated. Baumann and Gully (4) believed that the process is purely physico-chemical; they explained the acid nature of humus compounds as due to their ability, as colloidal bodies, to adsorb the bases and liberate the acids free.

More recently, however, the earlier chemical ideas have been prevailing. According to Heimann (8), for example, base absorption by "humic acids" is a stoichiometric relation; about as much base was found to be absorbed by freshly precipitated material as by the dry powder. The same kind of barium humate was prepared by precipitating an aqueous solution of the sodium salt (humate) with $BaCl_2$ as by treating the insoluble "humic acid" with $Ba(OH)_2$ solution. The salts of the "humic acids" thus prepared were found to possess a high base-exchange capacity, combining in the following manner:

$$H\text{-humate} + \text{Base} = \text{Base-humate} + H^+$$

Humus is a colloidal complex or a series of colloidal bodies, which possess a large surface and which show the characteristic properties of surface adsorption; this has no bearing, however, upon the binding of the bases. According to Aarnio (1, 2), the ratios between humus, on the one hand, and the iron and aluminum with which it combines, on the other, vary considerably, depending on the humus source and on the concentration of the bases, so that the Fe_2O_3: humus, for example, was found to range from 1:0.9 to 1:2.79. In the case of soils with a high humus content, the tendency is for a low Fe_2O_3 and a high Al_2O_3 content.

Since most soils contain an organic and an inorganic colloidal fraction, both of which are able to absorb bases, it is important to establish the relative functions of the two. Hissink (9) suggested that the organic portion of the soil has a much greater base-absorptive power than the inorganic or clay fraction; in estimating the lime requirement of the soil, he allowed for the humus fraction five times as much base-combining capacity as for the clay fraction. According to Alben (3), humus has seven times as much base absorptive capacity as bentonite. Sokolovski (22) calculated that, in the case of podzol soils, humus accounts for about two-thirds of the total absorptive capacity. Gedroiz (7) also found that the calcium absorption of the soil is due largely to its humus content. He stated that the importance of the organic or the inorganic soil fractions in this process varies with different soils and with different horizons of the same soil. In the case of a tchernozem soil with a humus content of 10 per cent, the base exchange capacity was 54.1 m.e., of which 26.1 m.e. was due to the inorganic fraction and 28.0 to the organic. Thus 100 gm. of the mineral part of the soil had 29 m.e., whereas 100 gm. of humus had 280 m.e. Since it is not the whole humus or the whole mineral part of the soil which acts as the absorption complex, but only 25 per cent of the mineral part and 30 per cent of the humus, in the particular soil, Gedroiz calculated that the mineral absorbing complex has a base-exchange capacity of 104.4 m.e. and the humus-absorbing complex of 933 m.e. or nine times as much.

Gedroiz suggested that the combination between the organic and inorganic soil colloids in the base-absorbing complex is not merely mechanical but is more intimate; this was demonstrated experimentally by Demolon and Barbier. According to Tiulin, the base absorption capacity of the soil, measured in terms of calcium, is parallel to the humus content, measured as total carbon. The carbon in the base-absorbing complex was thus found to be in constant relation to the soil absorptive capacity, this ratio being 2.7 for the tchernozem soils This ratio was much less for podzol soils, namely about 1.2, since, in addition to the humic-alumino-silicic complex, these soils contain also pure alumino-silicic compounds.

McGeorge (15, 16) found that the base-exchange capacity of mineral soils rarely exceeds 50 m.e. per 100 gm. of soil; however, in the case of organic soils, it reaches nearly 200. McGeorge, therefore, concluded that the organic matter of the soil functions largely as the exchange compound; he also found a chemical equivalent for the exchange reaction. McGeorge, as well as Tiulin (23), obtained a linear function for the ratio between the carbon content and the base-absorbing capacity, but not between the nitrogen content of the soil and the absorbing power. McGeorge believed these results to indicate that the non-nitrogenous compounds of the soil, largely the lignins and carbohydrates, are the principal absorbing agents. However, his conclusions that the nitrogenous constituents of the soil humus do not take any part in the absorbing capacity of the soil is totally unjustified, since he compared different soils with greatly varying C:N ratio, in which the chemical nature of the humus varied considerably.

According to Kotzmann (14), humus (prepared by Grandeau's procedure) combines with bases both chemically and physically. He obtained an equivalent weight of 308.7 for the ammonia absorbed and a similar Ca equivalent. By treatment of 1 gm. of "humic acid" with varying concentrations of bases, Kawamura (12) found that, as long as the initial concentration of the base does not reach about 3.0 m.e. per 100 cc., it is taken up completely. The humus thus formed absorbs a further amount of base, as the concentration of the latter increases. $AlCl_3$ was shown to form with "humic acid" a compound corresponding to Al-tri-humate.

In order to understand the origin of the bas-exchange capacity of the soil humus, it is necessary to consider the origin of the humus itself and its chemical nature. It was definitely established in previous contributions from this laboratory, as well as by others, that lignin is an important constituent of the soil humus. Lignin does not exist, however, in the humus in a free state, but is combined, physically or chemically, with other organic compounds to form larger complexes. One such complex was synthesized in this laboratory; it consisted of lignin and protein; it possessed characteristic properties which made it similar to the "humic acid" commonly isolated from peat or soil. Since lignin is the characteristic constituent of this complex, it is of importance to determine its reactivity with bases.

Wedekind and Garre (27) found that on treatment of lignin with different bases, a linear relation is obtained, the bases being irreversibly bound; this led to the conclusion that a true chemical compound has been formed. Kalb and associates (10) applied the term "alkali number" to designate the maximum absorption by lignin of base from one-half normal solution; 100 gm. of lignin absorbed 0.170 gm. equivalents of barium, corresponding to an equivalent weight of lignin of about 590. The fact should be recalled here that Oden (20) found that "humic acid" isolated from peat had a gram-equivalent of 330 and that Mattson (17, 18) obtained for the "humic acid" from peat a base-exchange capacity of 2.857 m.e., per gram, which shows an equivalent weight of 350. Wedekind and Garre also found that Cassel-brown or the natural "humic acid" behaves colloidally as lignin, and McGeorge has shown that the lignin content of organic matter and of highly organic soils is a linear function of the base-exchange capacity.

McGeorge found that lignin isolated from corn cobs had a base-exchange capacity of 18.6 m.e. of Ba and 20.6 of Ca per 100 gm., whereas "soil lignin" had 160.6 and 161.2 m.e. respectively. In view of the fact that the alcoholic sodium hydroxide extract of soil will contain other substances in addition to lignin, the term "soil lignin" can be applied to this fraction only with considerable reservation, since it is not equivalent to lignin which is usually obtained from plants. When the soil was extracted with aqueous NaOH solution, a substance was obtained ("humic acid"), which had a still greater base-exchange capacity, exceeding 300 (384–378). Lignin treated with alkali solutions or acids showed an increase in base-exchange capacity.

In a recent paper, Mitchell (19) demonstrated that the organic matter of different soils varies considerably in its base-exchange capacity; according to him, lignin freshly isolated from plant materials has a very low base-exchange capacity, whereas "lignin-humus," or the lignin of the soil, is largely responsible for the base-exchange capacity of the soil organic matter. The term "lignin-humus" or "soil lignin" was introduced by the senior author (26) to designate that constituent of the humus in peat and in mineral soils which has the chemical properties characteristic of lignin; it did not, of course, mean that this compound exists in the soil in a free state and independent of the other organic constituents of humus or that it can be isolated as such from it. Unfortunately, both McGeorge and Mitchell applied this term to that portion of the humus in peats and soils which is soluble in alkalies and is precipitated by acids, or which is not acted upon by 72 per cent sulfuric acid. A preparation thus obtained from peats, soils, and composts is not at all pure lignin; even if these preparations contain only small amounts of nitrogen, the latter is sufficient to change completely their properties from that of the original lignin, especially as regards the base-exchange capacity.

The isoelectric precipitation of humic acids from peat in the presence of proteins, on the one hand, and of iron and aluminum, on the other, has been recently investigated by Mattson. He found that the colloidal humic complex lowers the isoelectric point of the proteins. The "humates" of iron and aluminum show an electropositive maximum on the acid side of the isoelectric point, and when the proportion of humus is high, a second isoelectric point. The exchange capacity of humus, at pH 7.0, increased with the proportion of humus to the sesquioxide. The humate ion was found to displace the PO_4 ion and to a still greater extent the SiO_3 ion.

EXPERIMENTAL

It has been shown previously (24, 25) that lignin does not exist in humus in a free state but is combined with other substances to form various complexes which give the properties characteristic of humus. Among the most important substances which combine with lignin to give rise to some of the constituents of humus, the proteins or the organic nitrogenous complexes deserve first consideration. It has also been found that, as a result of interaction of lignins and proteins, complexes are formed which render the protein more resistant to decomposition by microörganisms. In view of the fact that lignin in a modified condition is no doubt responsible for some of the important physico-chemical properties of the humus, such as the base-exchange capacity, whereas in a free state it possesses only a comparatively low capacity of exchange, it remained to be determined how the complexes formed between lignin and proteins behave in this respect.

The determination of the base-exchange capacity of lignin and of ligno-protein complexes was carried out as follows: 1 to 1.5 gm. of the air-dry material or about 10 gm. of the fresh moist preparations were placed into 250-cc. beakers and treated with about 100 cc. of 1N calcium acetate solution of pH 7.0. The complexes were allowed to remain in contact with the acetate solution over night and then were transferred to filters and leached several times with the acetate solution until the last leachings were neutral in reaction. The complexes were then treated with about 10 cc. of 10 per cent $CaCl_2$ solution and washed with distilled water, at pH 7.0, till free from chlorides, as tested by the silver nitrate reagent. The calcium-saturated complexes, from which the excess of calcium salts has been removed were now leached with $1N$ NH_4Cl solution several times, in small lots, until the last leachings were free from calcium, as tested by ammonium oxalate. The leachings were concentrated to a small bulk and treated with ammonium oxalate solution; the precipitated calcium oxalate was now transferred to quantitative filter papers, washed free from chlorides, ignited, and weighed as CaO. From the weight of the oxide obtained, the base-exchange capacity of the complexes was calculated.

In the first two experiments, alkali lignin from rye straw and casein as a source of protein were used. Table 1 shows that the alkali lignin itself had only a low-base exchange capacity; however, the ligno-proteins, especially the calcium compound, had a high exchange capacity. No explanation can be offered at this time for this difference; it may be due to an insufficient saturation of the H-complex with calcium in the study of the base replacement. These results need further confirmation.

In order to determine the effect of protein concentration, three complexes containing varying amounts of casein were prepared. The ratio of lignin to casein was 2:1 (A), 4:1 (B), and 8:1 (C). The results (table 2) show that with a decrease in the relative protein content of the complex, the base-exchange capacity decreases. The exchange capacity of these complexes was compared with that of natural humus obtained from peat and mineral soil by extraction with 4 per cent NaOH solution and precipitation with mineral acid. It was found that the exchange capacity of humus varies considerably, depending upon the nature of the material from which it was extracted. The artificial humus complex prepared with a varying ligno-protein ratio had about the same capacity as the humus obtained from mineral soil, but less than the capacity of humus prepared from peat. The difference in the exchange capacity of the humus obtained from different sources may partly be due to differences in the chemical composition of the humus itself and partly to accompanying inorganic substances.

These results prove beyond doubt that the artificial humus complexes, or the synthesized ligno-proteins, possess a high base-exchange capacity, which places them on a level similar to that of the natural humus in soil. The results presented in table 1 point to the fact that the mineral part of the complex may have a most important influence upon the base-exchange capacity. In order to throw further light upon this problem, several 4-gm. quantities of the six preparations described in table 2 were dissolved or dispersed in a dilute alkali solution, the reaction adjusted with HCl to pH 7.0, and the complex precipitated with solutions of $CaCl_2$, $FeCl_3$, and $AlCl_3$. The precipitates

TABLE 1

The base-exchange capacity of lignin and of ligno-protein complexes

NATURE OF THE MATERIAL	BASE EXCHANGE CAPACITY IN M.E. PER 100 GM. OF MATERIAL
Alkali lignin	6.5
Hydrogen ligno-proteinate	63.2
Calcium ligno-proteinate	125.0

TABLE 2

Influence of protein concentration upon the base-exchange capacity of ligno-protein complexes

NUMBER OF COMPLEX	NATURE OF PREPARATION	RATIO OF LIGNIN TO PROTEIN	NITROGEN CONTENT	BASE-EXCHANGE CAPACITY, M.E. PER 100 GM. OF MATERIAL
			per cent	
1	Lignin	...	0.28	6.1
2	H-ligno-proteinate (A)	2:1	3.81	90.0
3	H-ligno-proteinate (B)	4:1	2.65	69.7
4	H-ligno-proteinate (C)	8:1	1.67	46.8
5	α-humus from peat	...	3.47	292.9
6	α-humus from soil*	...	2.79	73.2

* This preparation had 25.4 per cent ash, whereas the peat humus had only 4.0 per cent ash, showing that the first was not purified carefully from mineral constituents.

TABLE 3

Influence of base upon the base-exchange capacity of synthetic and natural humus preparations

NATURE OF PREPARATION	BASE USED FOR PRECIPITATION	NITROGEN CONTENT	ASH CONTENT	BASE-EXCHANGE CAPACITY, M.E. PER 100 GM. OF PREPARATION
		per cent	*per cent*	
Alkali lignin	Ca^{++}	0.28	5.8	17.9
Alkali lignin	Fe^{+++}	0.25	10.2	7.1
Alkali lignin	Al^{+++}	0.28	7.2	2.5
Ligno-casein complex, 2:1	Ca^{++}	2.77	9.3	148.5
Ligno-casein complex, 2:1	Fe^{+++}	3.14	9.2	35.6
Ligno-casein complex, 2:1	Al^{+++}	2.91	7.2	7.1
Ligno-casein complex, 4:1	Ca^{++}	1.82	11.2	123.6
Ligno-casein complex, 4:1	Fe^{+++}	2.58	7.4	23.6
Ligno-casein complex, 4:1	Al^{+++}	1.82	6.7	7.1
Ligno-casein complex, 8:1	Ca^{++}	1.37	7.7	90.0
Ligno-casein complex, 8:1	Fe^{+++}	1.68	7.0	22.1
Ligno-casein complex, 8:1	Al^{+++}	1.43	6.6	6.4
α-humus from peat	Ca^{++}	3.14	7.8	330.0
α-humus from peat	Fe^{+++}	3.36	12.1	84.7
α-humus from peat	Al^{+++}	3.02	8.7	32.1
α-humus from soil	Ca^{++}	2.18	30.8	135.9
α-humus from soil	Fe^{+++}	2.72	33.9	50.0
α-humus from soil	Al^{+++}	2.38	28.7	28.6

obtained were filtered off and thoroughly washed with distilled water. The calcium complee gave an invariably much more colored filtrate than the iron and aluminum complexes, pointing to a greater solubility of the former. The preparations were then dried, analyzed for nitrogen and ash, and used for the base-exchange determinations.

The results of the analyses of the base-exchange capacities of the various preparations given in table 3 tend to demonstrate that the nature of the inorganic ion combining with the organic substance is of the greatest importance in determining the base-exchange capacity of the preparation. They also point to an exact similarity in behavior beween the synthetic and the natural humus preparations. The lignin had a low base-exchange capacity, independent of the base with which it was combined, whereas the ligno-protein complexes had a comparatively high capacity. The calcium complexes showed the highest exchange of bases, and the aluminum complexes gave the lowest exchange. The humus isolated from peat again had a higher base-exchange capacity than the humus from mineral soils. In both cases, however, the nature of the base with which the humus was combined influenced markedly the base-exchange capacity of the humus. Both humus preparations behaved, however, in an exactly similar manner as the ligno-protein complexes. The influence of the sesquioxides on the cation exchange tends to confirm Mattson's results that the acidoid radical, in this case the artificial humus or ligno-protein complex, is in partial combination with the sesquioxide and that the free acidoid valencies are responsible for the base exchange.

It seemed important to determine to what extent the high base-exchange capacity of the synthetic humus preparations is due to the complex formation between the lignin and the protein, which results in a new compound having properties different from those of both constituent substances. This compound formed between lignin and protein has different chemical properties and exerts a specific effect upon the activities of microörganisms (25). It remained to be established to what extent the physico-chemical behavior of the complex, as measured by the base-exchange capacity, is different from that of lignin and protein individually.

For this investigation, gliadin prepared from wheat flour was used as a source of protein. A ligno-gliadin complex, of a ratio of 4:1, with hydrogen as the base, was prepared and used for the study of the base-exchange capacity. The results are reported in milli-equivalents per 100 gm. of the preparation. Lignin had a m.e. of 6.7, gliadin itself had a m.e. of 66.78, and the ligno-gliadin complex had a m.e. of 130.15. These results enable one to make the following calculations:

100 gm. of gliadin	66.78 m.e.
400 gm. of lignin	26.80 m.e.
500 gm. of mixture	93.58 m.e.
500 gm. of complex	650.75 m.e.
Gain in base exchange capacity	557.17 m.e.

The base-exchange capacity of the complex formed from lignin and gliadin is nearly seven times as great as could be accounted for by the two complexes individually. This gain is thus found to be due to certain new properties gained by the complex.

It will be shown in a later contribution that drying of the ligno-protein complexes makes them more resistant to decomposition by microörganisms. In order to determine whether these complexes vary in their base-exchange capacity depending on whether they are present in a freshly prepared moist state or in a dry state, a series of preparations containing different proportions of lignin and protein were made. Two proteins, namely, casein and egg-albumen, were used for this purpose. The lignin and the protein were dissolved in different proportions in alkali solutions, precipitated with HCl in the cold, and washed with distilled water until free from acid. A part of the moist preparations thus obtained was used immediately for base-exchange determinations, and another part was previously air-dried so as to reduce the moisture to about 6–8 per cent. The results are calculated in both instances on the basis of 100 gm. of dry material. For the purpose of comparison, the α-fraction was prepared from lowmoor peat, by extraction with alkali solution, precipitation with mineral acid and washing; this preparation as well was used, both in a moist and in a dry condition. It is to be recalled that the α-fraction is comparable to the "humic acid" preparations.

TABLE 4

Influence of drying of ligno-protein complexes upon their base-exchange capacity

NATURE OF PROTEIN	RATIO OF LIGNIN TO PROTEIN	NITROGEN CONTENT OF PREPARATION (DRY BASIS)	MOIST PREPARATIONS		AIR-DRY PREPARATION BASE-EXCHANGE CAPACITY, M.E. PER 100 GM. OF DRY PREPARATION
			Moisture content	Base-exchange capacity, m.e. per 100 gm. of dry preparation	
		per cent	*per cent*		
Casein	25:4	2.67	89.5	99.8	92.1
Casein	25:8	3.79	91.1	141.6	132.1
Casein	25:15	5.15	91.1	159.4	143.2
Egg-albumen	25:4	2.29	90.0	112.6	104.1
Egg-albumen	25:8	3.71	90.1	132.5	124.1
Egg-albumen	25:15	4.83	91.2	148.6	139.4
α-fraction from peat	.....	3.37	92.6	321.4	308.3

The results presented in table 4 show again that the base-exchange capacity of ligno-protein complexes increases with an increase in the relative protein content, not in direct proportion, however as is shown in figure 1. The process of drying of the complex reduces somewhat its base-exchange capacity, but not to any very marked extent.

The humus commonly used for the base-exchange determinations is obtained from peat or from mineral soil by extraction with dilute alkali solutions and precipitation by acids. It is usually assumed that this represents the colloid part of the soil humus which is most active in the absorbing capacity of the soil. It is quite essential to compare the base-combining properties of this fraction of humus with that of the humus as a whole as well as with the part left insoluble in dilute alkalies, namely, the "humin" fraction. A lowmoor peat was used for this purpose. Three kilograms of moist peat, containing 64 per cent moisture, was first extracted in the cold, with 4 per cent NaOH solution, for several days. The extract was removed by filtration through paper and the residue again extracted with fresh alkali solution in the cold. The filtrates were combined and treated with cold HCl. The precipitate formed was filtered off, washed with distilled water, dried, and labelled *1a*. The dry yield was 158 gm. The moist peat residue was now extracted with 4 per cent NaOH solution, for 1 hour at 15 pounds pressure. The extract was removed by filtration and the residue again extracted with fresh NaOH solution at 15 pounds pressure. The filtrates were combined and precipitated with HCl; the precipitate was washed and dried, yielding 100 gm. of dry material (labelled *2a*). The peat residue was neutralized with dilute HCl solution, washed with distilled water, and dried; the residue (labelled *3a*) weighed 180 gm. The base-exchange capacity of these three preparations was compared with that of the original peat. In addition to the lowmoor peat, a woody peat from Wisconsin, as well as the α-humus preparation from this

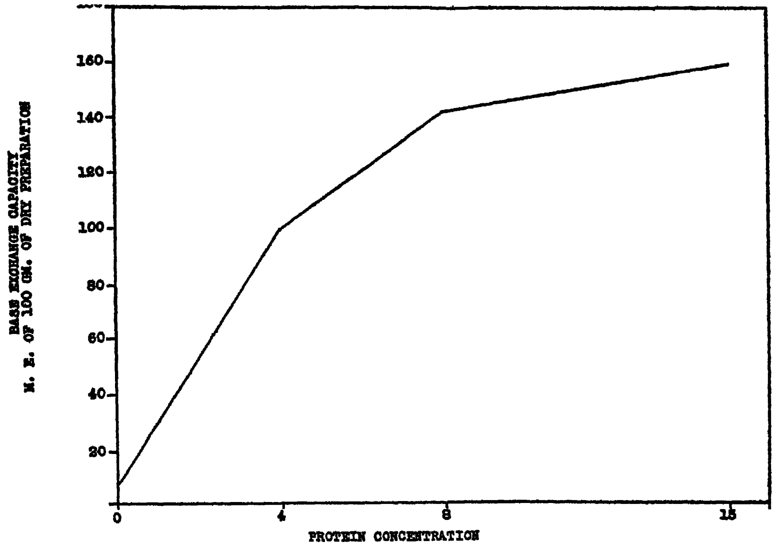

FIG. 1. INFLUENCE OF PROTEIN CONCENTRATION IN THE LIGNIN-PROTEIN COMPLEXES UPON THEIR BASE-EXCHANGE CAPACITY: PARTS OF PROTEIN TO 25 PARTS OF LIGNIN

peat obtained by extraction with hot NaOH solution, and Cassel Brown obtained from Germany were used.

The results given in table 5 show that the alkali extracts from peat a part of the organic matter which has a much higher base-exchange capacity than the original peat. However, the residual part of the peat, left after the alkali extraction, is not totally devoid of such capacity.

TABLE 5

Base-exchange capacity of different preparations from peat and Cassel Brown

NATURE OF PREPARATION	ASH CONTENT	NITROGEN CONTENT	BASE-EXCHANGE CAPACITY, M.E. PER 100 GM. OF DRY PREPARATION
	per cent	*per cent*	
Original lowmoor peat (a)	13.1	3.55	145.3
Cold alkali extract (1a)	2.5	3.26	234.3
Hot alkali extract (2a)	0.8	2 68	246.0
Residual "humin" (3a)	14.4	2.21	124.0
Woody peat (b)	17.8	2.37	118.0
Alkali extract from woody peat (1b)	2.6	3.16	251.3
Cassel Brown	11.6	0.68	252.0

SUMMARY

Artificial humus complexes, namely ligno-proteins, possess a much higher base-exchange capacity than does free lignin.

The base-exchange capacity of ligno-protein preparations increases with an increase in the protein content.

The nature of the base used in preparing the ligno-protein complexes influences considerably their base-exchange capacity, the calcium complex giving the highest capacity and the aluminum, the lowest; iron being a weaker base stands midway.

Natural humus preparations obtained from mineral soils and from peat showed distinct differences in their base-exchange capacity.

The base-exchange capacity of a complex formed from lignin and gliadin was about seven times as great as that of the individual constituents.

Drying of the ligno-protein complexes reduces their base-exchange capacity only to a limited extent.

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CONTRIBUTION TO OUR KNOWLEDGE OF THE CHEMICAL NATURE AND ORIGIN OF HUMUS: IV. FIXATION OF PROTEINS BY LIGNINS AND FORMATION OF COMPLEXES RESISTANT TO MICROBIAL DECOMPOSITION

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The majority of cultivated and uncultivated field and garden soils contain about 0.1 to 0.2 per cent total nitrogen, and forest, peat, and prairie soils contain even larger amounts of this element, which is so essential for plant growth; certain lowmoor peats contain as much as 3 per cent of nitrogen, on a dry basis. This element is present in the soils and in peats almost entirely in organic forms. Different methods of analysis have definitely established the fact that this nitrogen is largely protein in nature, since, on hydrolysis by acids, various amino acids are obtained (6, 7, 4, 5, 3). When calculated on the basis of the total organic matter of these soils, the protein is found to make up nearly 30 to 35 per cent (13). In spite of the fact that such large quantities of combined nitrogen are present in the soil, however, only small amounts of it are at any one time available to plant growth; usually not more than 1 to 2 per cent of the total nitrogen becomes available in a single growing season. When compared with the protein added to the soil in the form of plant and animal residues, such as cottonseed meal, linseed meal, dried blood, tankage, or alfalfa, it is found that the nitrogen of the soil is only very slowly made available through decomposition by microörganisms.

Numerous suggestions have been made to explain this resistance of the nitrogen in the soil humus to decomposition. As long as the origin of this humus, following the decomposition of plant and animal residues, and its chemical composition were not sufficiently understood, all attempts to explain the resistance of the nitrogen compounds to decomposition by microörganisms remained in the realm of guesswork and were not based on experimental evidence. More recently, considerable light has been thrown upon the origin of soil humus and even upon its chemical nature; one could now attempt to elucidate also the function of the nitrogen in the humus complex and its behavior in respect to microbial decomposition.

Among the facts which have been definitely established in regard to our recent knowledge of humus, the following must be emphasized:

1. Nitrogen forms an essential part of the soil humus; it is not an impurity, as assumed by many of the adherents of the "humic acid" hypothesis of the structure of humus, nor is it an

admixture; it is not absorbed by the humus from the atmosphere, but its origin in humus is due to processes which are responsible for the very formation of humus in soils and in composts.

2. Nitrogen accumulates in the form of organic compounds, during the process of the decomposition of plant residues, which result in the formation of humus. If the plant residues contain only a limited amount of nitrogen, as in the case of straw or corn stalks, the addition of available inorganic (or organic) nitrogen is essential to hasten their decomposition. During the process of decomposition, this nitrogen will be transformed by the microörganisms responsible for the decomposition processes into microbial proteins. A definite relation can be shown to exist between the extent of decomposition of the plant residues rich in carbohydrates and the synthesis of microbial protein. This information tends to emphasize the fact that, although some of the nitrogen in the soil humus may be of plant origin, a large part, if not most of it, is of microbial origin (8, 9).

3. In the course of decomposition of plant residues, the various carbohydrates, fats, and proteins were found to be readily decomposed, with different degrees of rapidity, depending on the nature of the residues, their chemical composition, and conditions of decomposition. The lignins, however, are most resistant to decomposition; hence they accumulate with the advance of decomposition. They accumulate more readily in soils saturated with water than in aerated soils, at low temperatures and acid reactions more readily than at high temperatures and at neutral or alkaline reactions (10). Some of the hemicelluloses also tend to persist in the soil humus; part of these at least is also due, as in the case of the proteins, to microbial synthesis.

4. The decomposition of plant and animal residues in soils, composts, and peat bogs accompanied by the formation of humus is thus characterized by the accumulation of lignins and the formation of proteins and to a less extent of certain resistant hemicelluloses and various other complexes.

The question then suggested itself: To what extent is the presence of the lignins responsible for the resistance of the proteins to decomposition; further, to what extent are these complexes combined chemically so as to produce humus with all its characteristic properties and reactions?

It was shown previously (11, 12) that lignin and protein form complexes which render the protein highly resistant to attack by microörganisms. These complexes possess the properties which are characteristic of the major portion of the soil humus, namely, that part which is soluble in alkalies and precipitated by acids, frequently spoken of as "humic acid" or "ulmic acid." The ligno-protein complexes were prepared by dissolving or dispersing the protein and the lignin in alkali solutions, mixing these and precipitating the complex either by an excess of hydrochloric acid, or by adjusting the reaction first to pH 7.0–7.5, then adding $CaCl_2$ solution to bring about the precipitation; the complex was then washed free from acid or salt, dried, and used for various chemical and biological studies.

A number of factors still remained to be studied. First, how much protein is the lignin able to combine with, thus making it resistant to microbial attack? Is there a definite proportion between the lignin and the protein in the formation of "humus" complexes, or does the ratio vary considerably? If an excess of protein is used, will the excess of protein be decomposed as readily as free protein or is it influenced by the presence of the ligno-protein complex? How does drying influence the behavior of the ligno-protein complex? To what extent does the complex have to be dried before it becomes resistant to decomposition by microörganisms?

Another very important question can also be raised here: To what extent does the natural humus of the soil bind proteins in a manner similar to the action exerted by the lignins? In a recent paper, Demolon and Brigando prepared a complex consisting of 30 per cent albumen and 70 per cent humus, with a total nitrogen content of 6.63 per cent. On comparing the decomposition of this complex in soil with that of the humus itself, containing 3.37 per cent nitrogen and isolated from artificial manure, they found that although the nitrogen in the humus did not nitrify, the nitrogen of the albumen fixed by the colloidal humus nitrified just as well as an equivalent amount of free albumen.

When these investigations had already been completed, there appeared a paper by Hobson and Page (3) as a part of a series of studies on the carbon and nitrogen cycles of the soil, in which the authors combined proteins (egg albumen) with lignin; preparations were obtained which resembled "humic acid," in regard to the action of proteolytic enzymes upon the protein constituents. The various methods of treatment of the ligno-protein complex and of natural "humic acid" showed that the nitrogen in the two behaved in a similar manner. They concluded that the "humic acid" of the soil consists of a non-nitrogenous "humic acid" and of a protein; the association between the two is more intimate than mere "salt" formation as a result of mutual precipitation in acid solution. The "soil humic acid" was believed to consist of a negatively charged acidic colloid or "humic acid" and positively charged protein, on the basic side of its isoelectric point. Hobson and Page accepted the older conception of the existence of "humic acids" in the soil as independent chemical entities and free from nitrogen. They would have been more justified in concluding that soil "humic acid" is an intimate complex of lignin (or modified lignin) and protein. As to the resistance of the protein in the ligno-protein and "soil humic acid" complexes to the action of proteolytic enzymes, such as pepsin and trypsin, it is of interest to call attention to the work of Boysen-Jensen (1), who found that with the advance in age and in decomposition of the marine plant *Zostera*, there is a decrease in the relative amount of nitrogen acted upon by pancreatic enzymes: in the case of the green plant, 23 per cent of the nitrogen is made soluble by the action of the enzyme; when the plant has become brown, only 7.7 per cent of the nitrogen is made soluble by the enzyme under similar conditions of action; in the case of the humus in the sea bottom, 6.6 per cent of the nitrogen is thus rendered soluble. This led Jensen to the conclusion that *Zostera* contributes primarily to the marine humus.

EXPERIMENTAL

The following experiments were undertaken primarily for the purpose of answering the foregoing questions, as well as to shed further light upon the nature of the ligno-protein complex, using different lignin and protein preparations.

The first experiment deals with the decomposition of ligno-protein complexes containing increasing amounts of protein. Alkali-lignin was used as a source of lignin, and casein as a source of protein. The method of preparation of the complexes was as follows: Three-gram portions of casein were dissolved in 24-cc. portions of 0.1 *N* NaOH solution and to these were added alkali solutions containing 1.5, 3.0, 6.0, and 12.0-gm. portions of lignin, thus giving preparations with the following ratios of lignin to protein: 1:2, 1:1, 2:1, 4:1. The mixed solutions were then treated with HCl until complete precipitation took place, an excess of acid and heat being avoided. The precipitates were filtered and washed with distilled water until practically free from acid. The preparations were then allowed to dry for 24 hours at room temperature, higher temperatures being avoided.

In a similar manner three other mixtures were prepared, using instead of lignin a ligno-casein complex with 2.1 per cent nitrogen. The various moist preparations, in quantities equivalent to 1 to 4-gm. portions of dry material, were then added to 100-gm. portions of soil, well shaken so as to distribute the complex in the soil, brought to optimum moisture for aerobic decomposition, placed in an incubator at 26°C., and connected with the respiration apparatus. The CO_2 liberated was determined and recorded as milligrams of carbon. At the end of 27 days' incubation, the ammonia and nitrate liberated in the soil were also determined, aliquot portions of soil being used; a portion of the soil was treated with 100 cc. 4 per cent alkali solution, and the extract precipitated with acid; the washed and dried precipitate was recorded as the α-fraction. The results are given in table 1.

The nitrogen content of the ligno-protein preparations varied from 3.66 to 10.16 per cent, while the total amount of nitrogen introduced into the soil in the form of these complexes varied from 82.7 to 214.8 mgm. An examination of the results of the decomposition of these preparations, as measured by the amount of carbon liberated as CO_2 and the nitrogen as ammonia and nitrate, will reveal the fact that the rapidity of the decomposition of the ligno-protein complexes is not parallel to the total amount of nitrogen added to the soil, but to the amount of free protein; the latter can be calculated by allowing for that amount of protein which is combined with the lignin. This can be brought out readily when the results of the decomposition of the ligno-protein complexes with a different ligno-protein ratio are compared with the decomposition of the free casein added to the same amount of soil. When 1 gm. of air-dry casein containing 160 mgm. of total nitrogen was added to 100 gm. of soil, there was liberated, during the period of decomposition, 307.6 mgm. of carbon as CO_2; during the same period there was accumulated 97.5 mgm. of mineralized nitrogen, above the control soil. When only 0.5 gm. of casein was used, the corresponding amounts of carbon and nitrogen were 170.4 and 37.4 mgm. In the presence of an excess of casein (1-gm. portions), all the nitrogen liberated accumulated as ammonia, while nitrate formation was completely repressed; with the smaller amount of casein, one-third of the nitrogen liberated was oxidized

TABLE 1

Influence of lignin: protein ratio in the ligno-protein complex upon its decomposition in soil and formation of humus

RATIO OF LIGNIN TO PROTEIN IN LIGNO-PROTEIN COMPLEX	$\frac{\text{LIGNIN}}{\text{PROTEIN}}$				$\frac{\text{LIGNO-PROTEIN}}{\text{PROTEIN}}$			CASEIN ALONE		LIGNIN ALONE	SOIL ALONE
	1:2	1:1	2:1	4:1	1:1	2:1	4:1				
Nitrogen content of complex, *per cent*	10.16	8.27	5.56	3.66	8.54	7.02	5.37	16.0	16.0	0.15	......
Amount of complex (dry basis) used per 100 gm. of soil, *gm.*	1.0	1.0	2.0	4.0	1.0	2.0	4.0	1.0	0.5	1.0	0
Total nitrogen added to 100 gm. of soil, *mg.*	101.6	82.7	111.2	146.4	85.4	140.4	214.8	160.0	80.0	1.5	0
Free nitrogen,* *mgm.*	89.6	67.5	75.7	74.2	67.3	105.1	134.6	160.0	80.0	......	0
Free protein,† *mgm.*	560	422	473	464	421	657	841	1,000	500	......	0
CO_2 liberated above control, *mgm. C.*	229.7	144.3	163.8	149.4	148.3	206.9	241.9	307.6	170.4	10.3	24.9
NH_3-N, *mgm.*	43.8	24.3	37.2	27.0	17.2	31.5	52.3	100.4	25.1	−0.3	0.9
NO_3-N, *mgm.*	16.9	12.7	−0.1	−2.9	6.7	9.5	−0.7	−2.9	12.3	−0.7	2.9
Total nitrogen above control, *mgm.*	60.7	37.0	37.1	24.1	23.9	41.0	51.6	97.5	37.4	−1.0	......
Humus in flask (α-fraction) above control, total, *mgm.*	515	760	1,457	3,539	374	1,097	2,800	Tr.	Tr.	959	681
Humus in flask (α-fraction) above control, nitrogen content, *per cent*	2.75	2.64	2.33	2.04	3.12	2.63	2.54	......	......	1.13	2 65
Ratio of nitrogen liberated to free nitrogen, *per cent*	67.5	54.8	49.0	32.5	35.5	39.0	38.3	61.0	46.8	......	0

* Allowing 2 to 3 per cent nitrogen for the ligno-protein complex resistant to decomposition, as shown by the nitrogen content of the α-humus fraction.

† Free protein = free nitrogen × 6.25.

to nitrate. The addition of free lignin to the soil brought about only a limited amount of decomposition, as shown by the increase of CO_2 evolution by 10.3 mgm. of carbon; this was accompanied by a decrease in the amount of mineralized nitrogen, which is no doubt due to the fact that the microörganisms bringing about the limited decomposition of lignin, a complex practically free from nitrogen, consumed a small amount of the nitrogen liberated in the decomposition of the soil humus.

In the case of the ligno-protein complexes, the largest amounts of total nitrogen introduced were 146.4 mgm. in the 4 gm. of the 4:1 ligno-protein complex, 140.4 mgm. in the 2 gm. of the 2:1 of ligno-protein-protein complex, and 214.8 mgm. in the 4 gm. of the 4:1 ligno-protein-protein complex. The average amount of nitrogen in these three preparations, 167.2 mgm., is greater than the amount of nitrogen introduced in the 1 gm. of free casein. The total carbon liberated as CO_2 in the decomposition of these preparations above the control soil was 149.4 mgm., 206.9 mgm., and 241.9 mgm., with an average of 199.4 mgm. This is much below the amount of CO_2 produced by the 1 gm. of casein (307.6 mgm.) and approaches more closely that liberated from 0.5 gm. of casein, namely, the 170.4 mgm. The corresponding quantities of nitrogen liberated as ammonia and nitrate in the decomposition of the three aforementioned preparations were 24.1, 41.0, and 51.6, with an average of 38.6 mgm., above the control soil. These as well were much lower than the nitrogen liberated from the 1 gm. of casein (97.5 mgm.) and were again similar to the amount of nitrogen liberated in the decomposition of 0.5 gm. of casein (37.4 mgm.). In other words, as a result of the decomposition of 2 to 4-gm. portions of the ligno-protein preparations, containing more than 1 gm. of protein, the products liberated as CO_2 and as available nitrogen were equivalent to the corresponding products liberated from only about one half as much free protein. The other half of the protein was intimately bound by the lignin, whereby the protein was made highly resistant to microbial attack.

At the end of the decomposition period, the various soils were treated with dilute alkali solutions and the filtered extract was precipitated with an excess of hydrochloric acid, thus giving the α-humus or "humic acid" fraction of the soil. The results obtained show a decided increase in this fraction as a result of the addition of the ligno-protein complexes; the data tend to add further weight to the interaction of lignin and proteins which result in the formation of humus in soil. The total increase in the α-humus content in the soils receiving the three aforementioned preparations was 3.539 gm., 1.097 gm., and 2.800 gm., with a corresponding nitrogen content of 2.04, 2.63, and 2.54 per cent. It is this nitrogen which was removed from the state of a free protein and became bound to the lignin group. By subtracting this nitrogen from the total nitrogen added in the form of the complex to the soil, one obtains a figure which can be spoken of as "free nitrogen." The ratio of the nitrogen liberated in an available form in the process of decomposition to this free nitrogen is very similar to the ratio of the nitrogen mineralized to the total nitrogen content in the form of free protein (casein) added to the soil. The average of all the ratios (free nitrogen: mineralized nitrogen) of the various ligno-protein preparations is 45.2, which is nearly the same as the ratio obtained for the decomposition of the 0.5-gm. portion of the casein.

The protein in the ligno-protein preparations with a higher nitrogen content, or with a narrower lignin:protein ratio, decomposed much more readily, as shown by the evolution of CO_2 and the liberation of nitrogen as ammonia and nitrate, than the protein in the ligno-protein preparations with a lower nitrogen content or a wider lignin:protein ratio. In the case of the 1:2, 1:1, and 2:1 ligno-protein preparations and the 1:1 ligno-protein-protein, with a nitrogen concentration of 10.16, 8.27, 5.56, and 8.54 per cent respectively and a total corresponding nitrogen content of 101.6, 82.7, 111.2, and 85.4 mgm., the decomposition was, as shown by the CO_2 liberation, 229.7, 144.3, 163.8, and 148.3 mgm. carbon, with an average of 171.5 mgm.; the corresponding nitrogen liberation was 60.7, 37.0, 37.1, and 23.9, with an average of 39.7 mgm. These

TABLE 2

Influence of peat humus: protein ratio in the humus-protein complex and its decomposition in the soil

RATIO OF PEAT-HUMUS TO PROTEIN IN HUMUS-PROTEIN COMPLEX	$\frac{\text{PEAT HUMUS}}{\text{PROTEIN}}$				CASEIN ALONE		PEAT HUMUS ALONE
	1:2	1:1	2:1	4:1			
Nitrogen content of complex, *per cent*	11.09	9.38	7.51	5.70	16.0	16.0	2.97
Amount of complex used (dry basis) per 100 gm. of soil, *gm.*	1.0	1.0	2.0	4.0	1.0	0.5	3.0
Total nitrogen added to 100 gm. of soil, *mgm.*	110.9	93.8	150.2	228.0	160.0	80.0	89.1
Free nitrogen,* *mgm.*	101.0	84.2	119.2	143.5	160.0	80.0	....
Free protein,† *mgm.*	631	526	745	897	1,000	500	....
CO_2 liberated above control, *mgm. C.*	243.6	198.1	214.6	172.4	307.6	170.4	19.0
NH_3-N, *mgm.*	39.9	40.5	51.6	54.1	100.4	25.1	3.6
NO_3-N, *mgm.*	16.7	9.6	2.3	−1.6	−2.9	12.3	0.2
Total nitrogen above control, *mgm.*	56.6	50.1	53.9	52.5	97.5	37.4	3.8
Humus in flask (α-fraction) above control, total, *mgm.*	279	789	1,127	3,200	Tr.	Tr.	2,884
Humus in flask (α-fraction) above control, nitrogen content, *per cent*	2.70	2.47	2.47	2.72	....	......	2.59
Ratio of nitrogen liberated to free nitrogen, *per cent*	56.0	59.5	45.2	36.6	61.0	46.8	....

* Allowing 2 to 3 per cent nitrogen for the ligno-protein complex resistant to decomposition, as shown by the nitrogen content of the α-humus fraction.

† Free protein = free nitrogen × 6.25.

results were almost exactly the same as the amounts liberated in the decomposition of the 0.5-gm. portion of casein in soil. Here as well, a certain amount of protein was bound, but was not as large as that bound by the larger concentrations of lignin. Where the lignin-protein ratio was narrow, a much greater relative proportion of the protein underwent decomposition by microörganisms.

In order to compare the behavior of free protein in the presence of ligno-protein complexes with that of free protein in the presence of α-humus obtained from peat, the following experiment may be reported here (table 2). A humus fraction was prepared from peat by alkali extraction and acid precipitation. This humus was combined with different amounts of casein, in proportions of 1:2, 1:1, 2:1, and 4:1, by mixing the corresponding alkali solutions and precipitating with HCl, avoiding an excess of acid. The precipitates were washed with distilled water and drained, and aliquot portions of the moist preparations were added to 100-gm. portions of soil and allowed to decompose under conditions similar to those reported in the previous experiment. The data for the decomposition of the free casein are taken from table 1, so as to give a basis for comparison. In the process of precipitation of the peat humus-protein complexes, not all the protein came down with the preparation, since some remained in solution. Hence, 4 gm. of the 4:1 humus-protein preparation carries less than 1 gm. of protein. The free nitrogen figures should therefore be used for these calculations. These figures were obtained by subtracting from the total nitrogen the amount of nitrogen in the α-humus fraction which has actually been found in the soil at the end of the experiment.

The peat humus alone decomposed only very slowly, as shown by the limited liberation of carbon as CO_2 (19.0 mgm.) and of nitrogen as ammonia and nitrate (3.8 mgm.). The humus-protein preparations, containing an average of 112.0 mgm. of free nitrogen, liberated 207.2 mgm. of carbon as CO_2 and 53.3 mgm. of nitrogen in an available form. These data can then be compared with the average results obtained in the decomposition of the two quantities of free casein, taking these as 100:

Free nitrogen used in the humus-protein preparations	93.33
Carbon dioxide liberated	86.70
Nitrogen liberated as NH_3 and NO_3	78.96

The results are close enough, for experiments of this type, to permit the conclusion, when allowance is made for the nitrogen introduced into the humus-protein complex in the peat humus, that the bound protein was practically all in a free state and underwent as active decomposition in the soil as the free protein itself. In other words, humus isolated from lowmoor peat is already nearly saturated with protein and does not bind any more, or only very little more, protein. This fully confirms the results obtained by Demolon and Brigando, who used different procedures but arrived at similar results.

In the two previous experiments, the preparations were used in a moist condition, without any previous drying. However, in the studies reported previously (10, 11), dry preparations were used. In order to determine what effect the drying of the complex has upon the rapidity of its decomposition, several of the preparations used in experiment 1 were dried at 70°C., then added to 100-gm. portions of soil and allowed to decompose for 26 days (table 3).

The results show that a certain definite change has taken place in the process of drying, which renders the protein in the preparations even more resistant to decomposition. However, the general principles observed with the moist preparations hold true here as well: the greater the protein content of the preparation, the greater is the relative amount of protein decomposed, as measured both by the evolution of CO_2 and by the liberation of the nitrogen in an available form. The preparation containing 2 parts of protein to 1 part of lignin behaved as if it contained just about one-half gram of free protein. With an increase in the ratio of lignin to protein or with a decrease in the relative amount of protein in the preparation, the rapidity of decomposition diminished rapidly.

TABLE 3

Influence of drying upon the rapidity of decomposition of ligno-protein complexes

1-gm. portions of the dried preparations used

NITROGEN CONTENT OF DRY COMPLEX	CO_2 GIVEN OFF		NITROGEN LIBERATED			α-FRACTION		
	Total	Above control	NH_3-N	NO_3-N	Total-N above control	Total	Above control	Nitrogen
per cent	*mgm. C*	*mgm. C*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
Control*	22.3	.....	0.5	2.5	....	552	...	3.55
5.56	27.5	5.2	1.5	3.6	2.1	1,251	699	3.58
7.02	84.3	62.0	8.5	13.6	19.1	1,202	650	3.52
10.16	169.1	146.8	34.8	29.6	61.4	892	340	3.22

* Soil alone.

It seemed desirable to study the effect of the relative concentration of protein in the ligno-protein complexes, using other lignin and protein sources. For the following experiment, alkali lignin prepared from cypress wood and powdered egg-albumen were employed. Two preparations were made, as follows:

I. Five grams of powdered egg-albumen was dissolved in cold water by stirring. The protein solution was then added to a dilute NaOH solution of 7.5 gm. of lignin. The two solutions were well mixed and adjusted with HCl to a pH of 6.5. Five grams of $CaCl_2$ dissolved in water was then added to precipitate the ligno-protein complex. The precipitate was washed with distilled water and dried. The yield obtained was 9.78 gm. with an ash content of 4.94 per cent and a nitrogen content of 5.36.

II. Five grams of egg-albumen and 20 gm. of lignin were dissolved and mixed as in preparation I. The solution was precipitated with 5 gm. $CaCl_2$, yielding 22.95 gm. of preparation with an ash content of 11.78 per cent and a nitrogen content of 2.62 per cent.

For the study of the decomposition of these complexes, a Sassafrass soil was used; this soil gave 5.71 per cent loss on ignition and contained 0.146 per cent nitrogen. One hundred-gram portions of the moist soil (20.5 per cent moisture) were placed in a series of long-necked flasks. Different amounts of the two preparations were then added and well mixed with the soil, moisture was adjusted to optimum, and flasks were connected with the aeration apparatus in the incubator. The cultures were aerated for 1 hour every day and the CO_2 evolved was determined. At the end of 26 days, the soil was removed and analyzed for ammonia, nitrate, and the α-fraction (table 4).

The ligno-albumen complexes decomposed very slowly, as compared with free albumen. The preparations which contained the higher amount of nitrogen decomposed more readily than those containing the lower amounts. In the case of the complexes with 2.62 per cent nitrogen, there was actually less nitrogen liberated than in the control soil. Most of the ligno-albumen preparations introduced into soil was recovered in the α-fraction of the soil humus at the end of the experiment.

TABLE 4

Comparative rate of decomposition of two ligno-albuminates

NATURE OF COMPLEX ADDED TO SOIL	COMPLEX		CO_2 GIVEN OFF		NITROGEN LIBERATED			α-FRACTION				
										Nitrogen in α-fraction		
Lignin: albumen	Nitrogen content	Amount of preparation used	Total	Above control	NH_3-N	NO_3-N	Total-N above control	Total weight of organic matter	Above control	Total-nitrogen	Above control	Relative concentration
	per cent	*gm.*	*mgm.* C	*mgm.* C	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
0	0	0	22.3	.....	0.5	2.5	.....	552	.....	18.6	....	3.55
3:2	5.36	1	28.7	6.4	0.5	3.4	0.9	1,229	0,677	35.0	16.4	2.85
3:2	5.36	3	40.8	18.5	0.5	5.2	2.7	2,467	1,915	78.3	59.7	3.17
4:1	2.62	2	30.2	7.9	0.4	1.6	−1.0	2,119	1,567	46 8	28.2	2.21
4:1	2.62	5	39.3	17.0	0.7	0.8	−1.5	5,189	4,637	93.3	74.7	1.80
Albumen alone	....	0.5	129.9	107.6	23.3	20.4	40.7	0,592	0,040	22.0	3.4	3.71

In order to illustrate further the effect of drying upon the decomposition of protein in the ligno-protein complexes, the results of the following experiment may be reported here. Lignin prepared from chestnut wood, by the alkali extraction procedure, and casein were used. A ligno-casein complex was obtained containing 2.82 per cent nitrogen. Thirty grams of the preparation was dissolved in 1,000 cc. of 2 per cent NaOH solution. The solution was distributed in 10 flasks and the complex in each flask precipitated with 15 cc. of 1:1 HCl solution. The various precipitates were removed by filtration through paper and washed with distilled water until practically free from acid.

The residues were allowed to drain for a few hours, then weighed; the average weight of the several moist preparations was 30.28 gm. The nitrogen content of the filtrates was 7.2 mgm., hence each precipitate contained 71.4 mgm. of nitrogen. Two of the precipitates were allowed to remain moist; two preparations were dried at 70°C., giving 2.67 gm. of residual material with 3.3 per cent moisture; two of the precipitates were dried down to contain 80 per cent moisture and two, to 50 per cent moisture. These various preparations were then added to 150-gm. portions of fresh Sassafrass soil and well shaken so as to distribute the material throughout the soil. Enough water was added to the various flasks so that all should have the same optimum moisture. The CO_2 given off for a period of 25 days was measured and, at the end of that period of time, the ammonia and nitrate contents were determined in aliquot portions of the different soils. The α-fraction was extracted from an aliquot portion of the soil by the use of 4 per cent NaOH solution and precipitated with HCl.

TABLE 5

Influence of degree of drying upon the decomposition of protein in ligno-protein complexes

MOISTURE CONTENT OF PREPARATION, PER CENT OF TOTAL	CO_2 LIBERATED		NH_3-N	NO_3-N	TOTAL N		α-HUMUS, ASH-FREE	
	Total	Above control			Total	Above control	Weight	Nitrogen content
	mgm. C	*mgm. C*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Soil alone	39.3	.....	0.45	4.35	4.80	.....	795	24.6
3 per cent	66.3	27.0	1.05	3.20	4.25	0.55	2,553	49.0
50 per cent	130.4	91.1	0.90	6.75	7.65	2.85	2,472	52 2
80 per cent	127.5	88.2	5.70	9.25	14.95	10.15	2,832	58.4
91.2 per cent	153.0	113.7	3.15	7.00	10.15	5.35	2,466	50 1
Mixture of 600 mgm. casein and 2.4 gm. lignin	192.1	152.8	29.85	7.00	36.85	32.05	2,736	58.7
Casein alone, 600 mgm.	198.3	159.0	25.95	32.00	57.95	53.15	860	28.5

The results given in table 5 show that the ligno-protein complex becomes highly resistant to decomposition when it is well dried down. There is a marked reduction in the rate of decomposition of the protein even when the complex is in a water-saturated state, but decomposition becomes slower with the increase in the degree of dryness.

The following experiment tends to emphasize further the importance of drying in rendering the nitrogen constituents of the ligno-protein complexes especially resistant to decomposition by microörganisms. Six 25-gm. portions of alkali-lignin were combined respectively with 4, 8, and 15-gm. portions of casein, and with 4, 8, and 15-gm. portions of egg-albumen, giving preparations with increasing nitrogen content. These were precipitated and washed as before. In a similar manner, the α-fraction from lowmoor peat was also dissolved in an alkali solution, washed, and precipitated with an acid. Two portions from each of the moist preparations (weighing about 30 gm.), equivalent to 3 gm. of dry material, were added to 150-gm. portions of soil, well shaken, and incubated for 30 days. In a similar manner, two other portions were air dried and used in that condition; the moisture content of the soil was adjusted

TABLE 6

Decomposition of different ligno-protein preparations, in a moist state

LIGNIN-PROTEIN RATIO*	NITROGEN CONTENT OF PREPARATION (DRY BASIS)	TOTAL NITROGEN ADDED PER FLASK	CO$_2$ LIBERATED		NH$_3$-N	NO$_3$-N	TOTAL N		α-FRACTION, ASH-FREE	
			Total	Above control			Total	Above control	Total weight	Nirtogen content
	per cent	*mgm.*	*mgm. C*	*mgm. C*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
25:4C	2.67	80.14	107.6	66.3	9.10	9.10	18.20	13.65	2,224	62.64
25:8C	3.79	113.82	126.7	85.4	29.40	11.90	41.30	36.75	2,090	67.71
25:15C	5.15	154.56	216.8	175.5	54.60	24.75	79.35	74.80	2,050	67.15
25:4E	2.29	68.58	100.9	59.6	4.54	8.40	12.94	8.39	2,426	59.38
25:8E	3.71	101.22	119.2	77.9	11.90	10.15	22.05	17.50	2,500	81.35
25:15E	4.83	144.90	158.7	117.4	37.80	14.00	51.80	47.25	2,514	88.87
α-fraction from peat	3.37	101.22	61.0	19.7	7.35	7.00	14.35	9.80	2,322	84.03
Control soil	....	......	41.3	.....	1.75	2.80	4.55	.....	313	.....

* C = casein, E = egg albumen.

TABLE 7

Decomposition of different ligno-protein preparations, in a dry state

3 gm. of dry preparation added to 150 gm. of soil

LIGNIN-PROTEIN RATIO*	NITROGEN CONTENT OF PREPARATION	CO$_2$ LIBERATED		NH$_3$-N	NO$_3$-N	TOTAL N		α-FRACTION, ASH-FREE	
		Total	Above control			Total	Above control	Total weight	Nitrogen content
	per cent	*mgm. C*	*mgm. C*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
25:4C	2.67	49.5	23.0	3.85	5.25	9.10	3.60	3,087	72.6
25:8C	3.79	50.3	23.8	4.20	5.95	10.15	4.65	2,580	92.0
25:15C	5.15	51.2	24.7	4.20	8.05	12.25	6.75	2,420	117.9
25:4E	2.29	55.5	28.9	2.80	5.25	8.05	2.55	2,730	57.3
25:8E	3.71	51.5	25.0	3.20	6.65	9.85	4.35	2,648	83.9
25:15E	4.83	65.7	39.2	3.15	9.45	12.60	7.05	2,520	119.0
α-fraction from peat	3.37	42.5	16.0	3.85	6.30	10.15	4.65	2,465	80.2
Control soil	....	26.5	....	1.45	4.05	5.50	....	368	10.3

* C = casein, E = egg albumen.

to optimum. The results of the decomposition of the moist preparations are given in table 6 and those of the dry preparations, in table 7.

The results of this experiment confirm fully the previous observations, namely, that there is an increase in the rate of decomposition of the protein, as shown by the CO_2 evolution and nitrogen liberation, with an increase in the relative protein content. When 1 part of protein is allowed to interact with 6 parts of lignin, comparatively little decomposition takes place, as shown particularly by the nitrogen liberation, which is similar to that formed by an equal quantity of "α-humus" obtained from peat. However, when the lignin-protein ratio is 25:15, there is considerable decomposition of the protein; but here as well, there is left in the soil a complex similar in every respect to soil "humus." When the ligno-protein complexes were allowed to dry out before addition to the soil, their decomposition was markedly reduced, as shown by the CO_2-evolution, nitrogen liberation in the form of ammonia and nitrate, and residual humus complexes determined as the α-fraction.

Protein is thus shown to form with lignin a series of complexes. The lower the relative protein content of the complex the less is it subject to decomposition and the less of the nitrogen is liberated; the greater the relative protein content the more of the protein will be attacked by microörganisms in the soil and the more of the nitrogen will be liberated as ammonia.

DISCUSSION

Proteins are capable of forming with lignins, in different proportions, complexes which render them more resistant to attack by microörganisms. The greater the protein content of the complex the more readily can it be decomposed and, *vice versa*, the greater the proportion of lignin to protein the less readily is the latter subject to decomposition. Whether all the protein molecules are combined directly with the lignin molecules, or whether some of the protein molecules are bound to the lignin molecules and some to other protein molecules, giving a chain compound, the further links being more readly attacked, remains to be determined.

There is no doubt that the formation of a ligno-protein is not an instantaneous process, but is gradual, resulting from the intimate contact of the lignin with the protein; it is markedly influenced by certain factors, such as drying, temperature, reaction, and presence of bases.

It is quite probable that the ligno-protein complex in the soil humus is bound closely with other complexes, both organic, especially the hemicelluloses, and inorganic, especially the aluminum silicates. Whether this union is chemical or physical in nature is difficult to determine and is really of little significance; the probability is that it is partly physical and partly chemical.

Although soil humus is usually considered to be the organic fraction of the soil, there is no doubt that in its undisturbed condition in the soil it contains various inorganic elements which are an integral part of the humus complex. Some of these elements, like phosphorus and sulfur, are as much a part of the organic complex as the carbon and nitrogen; some of the elements, like calcium, magnesium, potassium, and partly iron and aluminum are bound to the complex chemically, giving definite compounds; other elements, like silicon in the form of silicates, give absorption compounds with the soil humus.

In speaking of the "humus-nucleus" or the ligno-protein complex in the soil humus, one need not imagine, therefore, that this fraction exists independently of the other organic constituents of the humus or even of certain of the inorganic elements. A knowledge of this fraction is important only from the point of view of the origin of humus, its chemical composition, and the rôle of the protein in its formation and functions.

Ligno-protein complexes are subject to greater decomposition by microörganisms in a fresh than in an air-dry state; drying of these complexes renders them considerably more resistant to rapid decomposition.

The decomposition of the ligno-protein complexes in the humus depends upon the relative ratio of the lignin to the protein in the complex, upon the conditions under which this was formed, its combination with bases, the nature of the inorganic part of the soil, soil conditions, and the presence of specific microörganisms.

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HAWAIIAN PINEAPPLE FIELD SOIL TEMPERATURES IN RELATION TO THE NEMATODE HETERODERA RADICICOLA (GREEF) MÜLLER[1]

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This report on soil temperatures in Hawaiian pineapple fields arose through the initiation of some preliminary tests of soils under cellophane covers.[2] Wishing to pursue the subject further, the writer continued temperature readings as time and conditions permitted. Admittedly, no systematic plan of procedure was outlined nor were observations at any time allowed to interfere with other work. Because of the conditions under which the records were made, they are not as comprehensive as one would desire. Nevertheless, an accumulation of temperature data resulted which seems to be of significance agriculturally in pineapple production, especially in relation to the nematode, *Heterodera radicicola* (Greef) Müller. This is particularly true since the recent preparation of a paper by Hoshino and Godfrey (10) which contained the results of a series of laboratory tests on lethal temperatures of root knot larvae and eggs in water. They concluded that temperatures of 40°C. and above are critical for this species. Thus it appears that again we find an example of a purely academic, laboratory study which has a direct bearing on field conditions. This paper will give sample records of actual field temperatures, and some discussion, with the work of these authors in mind.

Since Hoshino and Godfrey have already reviewed the literature pertinent to lethal temperatures it seems unnecessary to duplicate their work, and reference to them for papers on this subject will suffice, except for two papers which it might be well to cite as illustrations of the differences in lethal temperatures that have been reported by various authors. Thus, Nelson (12) failed to kill all individuals in peony roots when the latter were subjected to hot water treatment for 1 hour at 46.1°C., for 30 minutes at 48.9°C., or at 51.7°C. for 10 minutes. It is possible that the roots tested did not become sufficiently heated throughout, for, according to Hoshino and Godfrey (10), larvae should be killed in 4 minutes at 46.1°C., and eggs within 15 minutes. Again, Newhall

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[2] Temperature readings, under cellophane covers, were suggested to me by Dr R. N. Chapman, director of the Experiment Station of the Association of Hawaiian Pineapple Canners.

(13) found that temperatures between 46.7 and 48.9°C. in ordinary loam soil should be maintained for 10 minutes in order to kill eggs and larvae. This checks more closely with Hoshino and Godfrey as they report 1 to 4 minutes for killing larvae and 5 to 15 minutes for killing eggs at these temperatures, when eggs and larvae are free from soil.

In addition to the aforementioned papers there are two which mention temperatures in relation to the use of mulch paper in the Hawaiian Islands. Larsen (11) described the methods of applying mulching paper in sugar cane fields. He emphasized the value of the paper in raising temperatures of the soil in relation to weed control but kept no temperature records. Stewart, Thomas, and Horner (19) reported temperatures at 4 inches depth in the soil covered by mulching paper in pineapple fields. Their article is devoted to agronomic features of increase in soil temperatures due to this covering over the pineapple beds.

LOCATION AND ENVIRONMENT

The data presented here were obtained on the property of the field experiment station at Wahiawa, except certain of the air temperatures which are thermograph records compiled by the Signal Corps of the United States Army, located at Schofield Barracks.

Both Wahiawa and Schofield temperature stations lie in the pineapple section of the Island of Oahu, separated by a distance of a little less than 2 miles. The elevation is approximately 900 feet. The land is rather flat and slopes gently toward the coast on the south or, more abruptly, toward the mountain range east of Wahiawa, and toward a second range a little to the west of Schofield Barracks. The land continues its upward trend to the northwest for 2 or 3 miles and then drops rather rapidly to the coast line.

Although this location is cooler than most of the coastal regions of the Island, no extremes of temperature are experienced. Summer temperatures never reach the intensity found on warm days in North America. This fact makes these records of considerable interest in comparison with those shown by Sinclair (17) in Arizona, Chapman, et al. (4) in Minnesota, and others. More or less clearly defined rainy seasons occur in fall and early spring months but showers and cloudy weather are common in the summer months as well. Indeed, the water supply is dependent upon very frequent precipitation in the rain forests of the mountains. Banks of clouds overhead, a source of beauty and charm in the islands, have a profound influence on soil temperatures under certain conditions.

Pineapple field topsoil is very fine, usually acid, and highly colloidal. Its depth usually is 10 or 12 inches, and beneath, is an impervious clay-like subsoil.

So much for the physical environment surrounding these tests, and factors which might influence data on temperatures. We may now turn to a consideration of the records obtained under these conditions. So far as possible, all temperatures were taken at 8 a.m., 2 and 4 p.m. The temperatures are not absolutely comparable as tables 6 and 8 were prepared from data taken at other times, for the most part.

CONSIDERATION OF DATA

Comparisons of air temperatures

Since we must depend upon Schofield Barracks air temperatures in some of these comparisons, it might be well to show the similarity between the two localities in this regard. Table 1 shows the air thermograph temperatures at Schofield and at Wahiawa, July 9 to 31, 1931. The temperature at Wahiawa was warmer by 1.04 ±0.30°C. at 8 a.m., and at 4 p.m. was 1.09 ±0.22°C. colder than Schofield. Similar readings taken from September 9 to October 26, 1931 (also table 1) show that Schofield again is warmer at 4 p.m. by 0.95 ±0.13°C. Other differences are within the limits of probable error and altogether it is apparent that, for our purposes, readings from Schofield may be employed where Wahiawa air temperatures are lacking.

TABLE 1

Comparison of average air temperatures at Schofield Barracks and Wahiawa, recorded by*

TIME	JULY 9–31, 1931		SEPTEMBER 9–OCTOBER 26, 1931	
	Wahiawa	Schofield	Wahiawa	Schofield
	°*C.*	°*C.*	°*C.*	°*C.*
8 a.m.	25.04 ±0.26	24.00 ±0.15	24.60 ±0.12	24.75 ±0.80
2 p.m.	26.85 ±0.20	27.55 ±0.18	25.84 ±0.11	25.75 ±0.11
4 p.m.	25.21 ±0.18	26.30 ±0.14	23.85 ±0.09	24.80 ±0.10

* These records are made available for publication through the courtesy of Major General Albert J. Bowley, Commanding Officer of Schofield Barracks.

TABLE 2

Comparisons of average temperatures of air by thermometer in sun and thermometer in shade; air with housed thermograph; uncovered soil at surface, ½ and 3-inch depths, January 18–31, 1931

CONDITIONS OF READINGS	8 A.M.	2 P.M.	4 P.M.
	°*C.*	°*C.*	°*C.*
Air, thermometer, sun	20.81 ±0.22	24.00 ±0.51	22.55 ±0 40
Air, thermometer, shade	20.81 ±0.18	23.63 ±0.43	22.15 ±0.32
Air, thermograph, housed	21.95 ±0.28	22.65 ±0.27	21.26 ±0.21
Air, soil surface, sun	21.18 ±0.23	28.77 ±0.90	26.00 ±0.73
Soil, ½ inch depth, sun	20.36 ±0.26	32.40 ±1.24	27 80 ±0.79
Soil, 3 inches depth, sun	19.54 ±0.17	23.77 ±0.31	24.50 ±0.38

As ordinary centigrade thermometers were used in most cases it might be wise, also, to indicate the degree of accuracy which was obtained through their use. In table 2, air temperatures are recorded both by means of thermometers and thermograph. All were elevated three or four feet above the ground. At 8 a.m. the readings were almost identical, but the thermograph reading was slightly higher. Thermometers in the shade and the sun were both very slightly higher at 2 and 4 p.m. than the thermograph in the thermograph housing, which was, of course, exposed to the sun. All of the differences are within the limits of probable error. These are winter temperatures and it is quite possible that summer differences would be somewhat greater, though perhaps not by statistically significant amounts.

Temperatures fluctuate comparatively little during the day. Tables 2, 3 and 5 show differences of not more than 3.7°C. between morning and maximum afternoon average readings for either summer or winter months.

Contrary to temperate climates, no great seasonal fluctuation in temperatures is evident. Tables 2 and 3 show that the differences in temperature have varied less than 10 degrees from summer to winter in the averages of the readings taken.

TABLE 3

Comparisons of average temperatures of air, thermograph, with uncovered soil at surface, ¼- and 3-inch depths, July 9–31, 1931

TIME	AIR	SOIL SURFACE	SOIL, ¼ INCH	SOIL, 3 INCHES
	°C.	°C	°C	°C
8 a.m.	25 04 ±0 26	32 52 ±0 81	29 43 ±0 56	25 84 ±0 20
2 p.m.	26 85 ±0 20	45 00 ±1 90	43 47 ±1 13	33 31 ±0 55
4 p.m.	25 21 ±0 18	37 34 ±0 81	38 41 ±0 73	34 13 ±0 51

Uncovered soil

These readings were taken at different depths in soil which was covered by no vegetation or artificial covering. They represent conditions existing in fallow pineapple fields surrounding the Wahiawa station.

When the thermometer lay uncovered on the soil surface, January 18 to 31, 1931, the average temperature was less than the soil at ¼-inch depth (table 2). This is the reverse of conditions in summer months, as shown in table 3. Presumably this condition was due to the shading effect of clouds, for the days were cloudy during this period and frequently there were light showers. This relationship between clouds and surface temperatures is well illustrated also in figure 1, during the latter part of July, 1931. For these few days clouds and occasional showers brought a sharp decline in all soil temperatures but affected air temperature least.

Uncovered soil at ¼-inch depths rose to temperatures between 20.5 and 38°C. in January, 1931, with an average of 32.4 ±1.24°C. at 2 p.m. September 9 to October 9, 1931, temperatures (table 4) were intermediate between winter and summer readings. Here maximum daily temperatures frequently exceeded

40°C. for more than 2 hours, with averages of 38.71±0.89°C. at 2 p.m. and 35.33 ±0.84°C. at 4 p.m. July, 1931, records (table 3) show averages of 43.47 ±1.13°C. at 2 p.m. and 38.41 ±0.73°C. at 4 p.m. Like the soil surface temperatures, they all fell off rather rapidly in the late afternoon.

At a depth of 3 inches, temperatures continue to rise all day, and the maximum is not reached by 4 p.m. During the winter months, in periods of cloudy and rainy weather, these averages often do not exceed 25°C., as shown by table 2 for such conditions, but figures 1 and 2 make it certain that individual daily temperatures between 38 and 40°C. occasionally are reached at this depth for periods of 2 hours or more, from July to November, at least.

Soil covered with mulch paper

Mulch paper, usually 36 inches wide, generally covers the pineapple bed. The interspace is uncovered. In field practice an interval of from 1 to several weeks intervenes between the time of laying the paper and the planting of pineapples. The planting material is set out by means of a flat dibble thrust through the paper into the soil. Soil covered by paper in the usual manner was tested for temperature records.

TABLE 4

Comparisons of average temperatures of air with uncovered and mulch paper covered soils at ½- and 3-inch depths, September 9 to October 9, 1931

TIME	AIR THERMOGRAPH	UNCOVERED SOIL		SOIL COVERED WITH MULCH PAPER	
		½ inch	3 inches	½ inch	3 inches
	°C.	°C.	°C.	°C.	°C.
8 a.m.	24.36 ±0.16	26.73 ±0.26	23.89 ±0.14	27.39 ±0.40	26.47 ±0.14
2 p.m.	25.51 ±0.13	38.71 ±0.89	29.36 ±0.33	42.69 ±0.50	32.80 ±0.44
4 p.m.	23.70 ±0.11	35.33 ±0.84	30.00 ±0.33	38.83 ±0.53	34.33 ±0.43

At a depth of ½-inch, mulch-covered soils exhibit the characteristic temperature curve of uncovered soil (table 4), but the temperature is materially increased beneath the paper. From September 9 to October 9, 1931, the average temperature at 2 p.m. was 42.69 ±0.50°C., and at 4 p.m. 38.83 ±0.53° C. The individual soil temperatures very frequently rose above 40°C. for more than 2 hours. Air temperatures taken at this time were registered by thermograph and averaged 25.51 ±0.13°C. and 23.70 ±0.11°C. respectively. Thus, temperatures of the soil, covered with mulch paper, at this depth exceeded air temperatures by 17.18 and 15.13°C. at 2 and 4 p.m. Compared with uncovered soil there is a gain in temperature of 3.74°C. during this test.

Temperatures at a depth of 3 inches do not tend to fall off at 4 p.m., but the maximum is reached after that time, as table 4 indicates. Although the September–October, 1931, records show only one individual temperature above 40°C., this temperature must be attained at frequent intervals, since records for uncovered soil, at other times, have reached 38 or 39°C. on several occasions (fig. 1). The period between October 13–23, 1931, shows temperatures of 40°C. on two afternoons, and 39 or 39.5°C. was reached on three other days (fig. 2).

Again a very interesting observation can be made regarding the effect of clouds on these readings. Most of the days from September 9 to October 9, 1931, were cloudy, but only a few records were taken under these conditions during the period October 13–23, 1931. In the first case the difference between uncovered soil and soil covered with mulch paper, at ¼-inch depth, was 3.74°C. During the second set of records, when the sky was clear most of the time, the difference between uncovered and mulch paper covered soils at ¼-inch depth was 7.08°C. and at 3 inches, 6.66°C.

Temperatures of the soil at a depth of 4 inches have been reported by Stewart, Thomas, and Horner (19). Through the courtesy of the editor of *Soil Science*, their records for one week in July were used in constructing table 5. Under mulch paper the temperature averages 34.28 ±0.50°C. at 2 p.m., and 33.57±0.40°C. at 4 p.m. Individual daily maximum temperatures frequently exceed 90°F. in the warmest months with an excess of 10° to 19°F. over air temperatures. The highest single temperature for the week was 98°F. (36.7°C.) for this depth.

TABLE 5

Comparisons of average temperatures of air with uncovered and mulch paper covered soils at 4 inches depth, thermograph readings

Data from Stewart, Thomas, and Horner (19), July 23–29, 1926

TIME	AIR	SOIL AT 4 INCHES DEPTH	
		Uncovered	Mulch paper
	°C.	°C.	°C.
8 a.m.	24 50 ±0 29	22.62 ±0.16	25.75 ±0 28
2 p.m.	28.14 ±0.21	27.28 ±0.32	34.28 ±0 50
4 p.m.	26.85 ±0.25	28.57 ±0.40	33.57 ±0 40

Cellophane covered soil

A few tests were made in which a wood frame, 22 by 14 by 1⅝ inches, was set firmly into the soil so that no air currents could pass under the edges. Over the top a sheet of thin cellophane paper was glued. Thus, a dead air space was formed which had a depth of approximately ¼ inches over the soil surface. Thermometers were thrust diagonally into the soil at the edge of the frame so that the bulbs came near the center of the dead air space at the required depths.

A warm period in the fall of 1930 (table 8) showed that the temperature at 2:30 p.m. averaged 68.95 ±1.41°C. at ¼-inch depth. The 3-inch reading was 38.13 ±0.54°C. at 2:30 p.m., and the air temperature, registered by thermograph at Schofield, was 27.70 ±0.17°C. at 2 p.m. Temperatures very frequently exceeded 40°C. but the maximum individual temperature recorded for the 3-inch depth was 46°C., which is lethal for certain insects also, according to Chapman (3).

TABLE 6

*Comparisons of average temperatures of air with uncovered soil, mulch paper covered soil, and parchment paper covered soil at ½-inch depth**

TIME	AIR	SOILS AT ½-INCH DEPTHS		
		Uncovered	Mulch paper	Parchment paper
	°C.	*°C.*	*°C.*	*°C.*
6 a.m.	18.25 ±0.15	18.40 ±0.23	18.91 ±0.20	19.32 ±0.24
12 noon	22.70 ±0.20	28.62 ±0.54	30.04 ±0.67	33 63 ±0.91
9 p.m.	18.50 ±0.16	19.40 ±0 26	21.33 ±0.33	22.00 ±0.21

*Unpublished data, Misc. Agr. Exp., 22, recorded by Messrs. W. A. Wendt and H. O. Thompson, November 21 to December 31, 1927.

TABLE 7

Comparisons of average temperatures of air, thermograph, with cellophane covered soil at ¼- and 3-inch depths, July 9–31, 1931

TIME	AIR THERMOGRAPH	CELLOPHANE COVERED FRAMES	
		Soil, ¼ inch	Soil, 3 inches
	°C.	*°C.*	*°C.*
8 a.m.	25.04 ±0.26	38.27 ±1.25	28.50 ±0.26
2 p.m.	26.85 ±0.20	66.66 ±2.35	39.81 ±0.73
4 p.m.	25.21 ±0.18	59.56 ±1.83	41.15 ±0.72

TABLE 8

Comparisons of average temperatures at 11 a.m., 2 and 3 p.m., Schofield Barracks air thermograph readings, with cellophane covered soil at ½- and 3-inch depths at Wahiawa, 11:30 a.m., 2:30 p.m., August 6 to September 18, 1930

TIME	AIR SCHOFIELD	CELLOPHANE COVERED FRAMES	
		Soil, ½ inch	Soil, 3 inches
	°C.	*°C.*	*°C.*
10:30 a.m.		59.98 ±1.02	31.78 ±0.27
11:00 a.m.	28.20 ±0.95		
2:00 p.m.	27.70 ±0.17		
2:30 p.m.		68.95 ±1.41	38.13 ±0.54
3:00 p.m.	27.10 ±0.16		

The presence of moisture in the soil has a very important bearing upon the temperatures attained. July is normally drier than any of the later summer or winter months. The records of air and soil temperatures for July, 1931, while cooler for air, showed higher soil temperatures. Air temperatures varied between 25 and 27°C. on the average (table 7). At the same time the soil at ½-inch depth under the frame was less than the ½-inch depth average for the preceding fall (table 8). At the 3-inch depth the average temperature was 39.81 ±0.73°C. at 2 p.m. and 41.15 ±0.72°C. at 4 p.m. At this depth there was a steady increase in temperature and from about 2 to after 4 p.m. the temperature often exceeded 40°C.

DISCUSSION

Temperature decline

The readings for the day ceased at 4 p.m. for all of the records except those shown in table 6, which has been prepared from unpublished data collected by Messrs. W. A. Wendt and H. O. Thompson, agriculturists of the experiment station. Consequently, only general statements can be made about the decline in temperatures in late afternoon. Figure 2 shows the maximum temperatures daily for a short period in October, 1931. These readings were taken at half-hour intervals from 2 to 4 p.m. The air temperatures generally attained their highest values at 2 or 2:30 p.m., whereas covered and uncovered soils at a 3-inch depth reached their peak usually at 3 p.m. Table 6 reveals the fact that even the subsurface temperatures, while dropping, had not yet given up the excess over air temperatures by 9 p.m. Nevertheless, all accumulated heat is lost before morning, from depths recorded by the thermometers, and each day builds up its own accumulation. It is probable that temperatures at deeper levels would give up heat more slowly. Indeed, Stewart, Thomas, and Horner (19) found that temperatures at a 4-inch depth were lost very gradually during the night until early morning temperatures penetrated to that depth, when they again started to rise, but there was no further accumulation of heat from day to day.

Comparisons of daily temperatures and wind velocity

Figure 1 was constructed to show the fluctuation in daily temperatures for air, uncovered soil, and cellophane covered soil. Below is a record of wind velocities from Schofield, for purposes of comparison with these fluctuations. From several such comparisons no apparent relationship can be found to exist between wind velocity and temperature change, unless it be true that temperature increases with greater movement of air currents, for the breeze increases in velocity in the afternoon when temperatures also are higher. Above the graph is a record of the weather conditions when the readings were taken. Cloudy weather is indicated by *C*; partly cloudy, by *P*; rain is shown by *R;* and sunshine is labelled *S*. Here we perceive the direct effect of obscuring the sky and how little wind velocity has to do with temperatures under these conditions. On continuously cloudy or rainy days the soil temperatures are lower. One stormy week occurred during the month and the graph clearly

FIG. 1

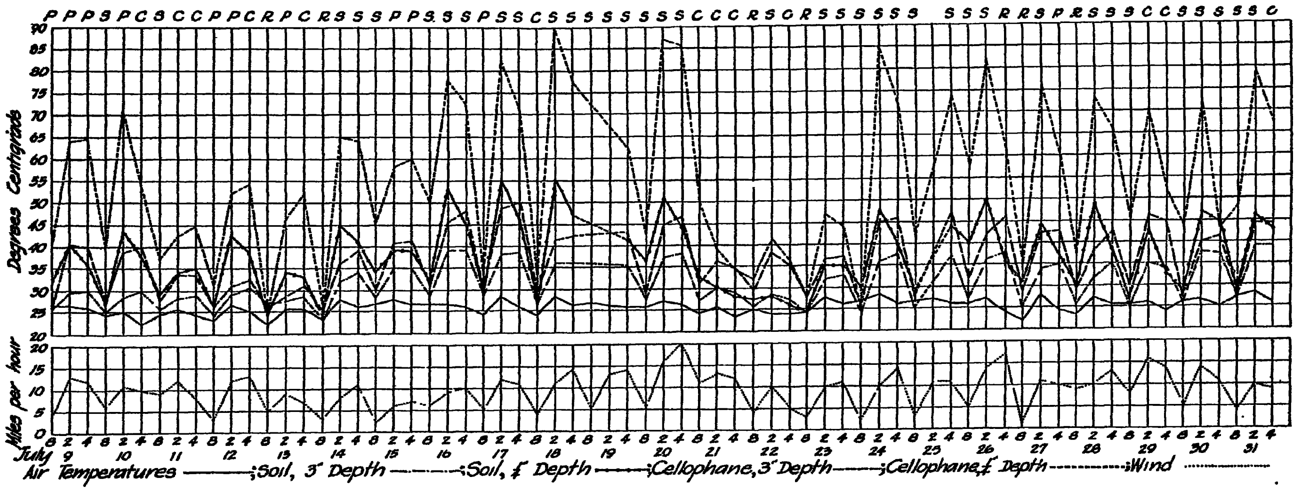

FIG. 2

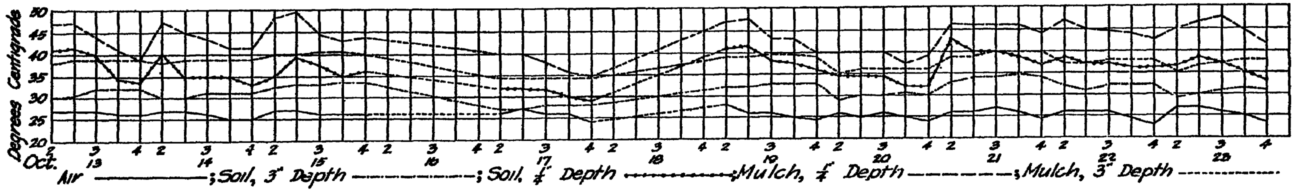

FIG. 1. DAILY FLUCTUATIONS IN TEMPERATURES OF AIR, OF UNCOVERED SOIL AT ¼- AND 3-INCH DEPTHS, AND OF CELLOPHANE COVERED SOIL AT ¼- AND 3-INCH DEPTHS

Weather conditions recorded at top: *C*, cloudy; *P*, partly cloudy; *R*, rain; *S*, sunshine. Beneath is shown wind velocities in miles per hour at the same time intervals. July 9 to 31, 1931 (except July 19, two readings; July 25, the 2 p.m. reading).

FIG. 2. FLUCTUATIONS IN PEAK TEMPERATURES AT HALF-HOUR INTERVALS FOR AIR, FOR UNCOVERED SOILS AT ¼- AND 3-INCH DEPTHS, AND FOR MULCH PAPER COVERED SOILS AT ¼- AND 3-INCH DEPTHS

October 13–23, 1931 (except October 16, 18) shows the effect of cloudy weather on all temperatures of the soil, which dropped abruptly. Air temperatures were affected very little, and wind velocities, too, continued about the same.

Root knot nematode and soil temperatures

Hoshino and Godfrey (10) recently have shown the maximum temperatures endured by the nematode, *Heterodera radicicola* (Greef) Müller, and the thermal death points, in relation to time, beyond which all larvae and eggs are killed. At 40°C. some eggs may survive for 4 or 5 days, but all larvae are killed in 2¼ hours. Lethal curves incline sharply with slight increases in temperature, and at 43°C. eggs will not survive more than 100 minutes or the larvae more than 8. One additional degree of temperature cuts the time for eggs to 50 minutes and the larvae to 6 minutes, before all are dead.

It has frequently been observed in preceding pages that subsurface temperatures of soil, whether covered or uncovered, average 40°C. for 2 hours or more at the ¼-inch depth. It is quite safe to conclude then, that the topmost portion of the soil is comparatively free from the larvae of this nematode where shading of the soil has not occurred. Individual temperatures of uncovered soil (fig. 2) sometimes attain 48°C. for shorter periods of time; sufficiently long, however, to kill all eggs too, for they cannot survive more than 10 minutes at this exposure, according to these authors (10).

Occasionally, during the summer months at least, the temperatures of the uncovered soil at a 3-inch depth reaches 38 to 39°C. (fig. 1). It has been shown that mulch paper causes an increase of 3.5 to 7°C. in temperature over uncovered soil at this depth, and these figures agree very closely with data obtained at a 4-inch depth by Stewart, Thomas, and Horner (19). It is therefore probable that lethal temperatures under paper extend down to the 3-inch level frequently enough to kill all larvae in the topsoil to this depth. The records show, too, such high temperatures even at a 4-inch depth that, while alive, the nematode population must be much reduced through the survival of only the hardier individuals, or else they are caused to migrate to lower levels. At any rate, their normal activities are much curtailed when such temperatures prevail.

From this it follows that plowing fields is beneficial from the nematode-control viewpoint. Frequent turnover of the soil exposes fresh soil in which the larvae, and many of the eggs, will be killed in the first few inches. Some additional data, not given here because incomplete, show that the soil at the top of a ridge, such as a plow furrow, is warmer than the bottom soil by 4.32 ±0.80°C., probably because both sides of the ridge, as well as the top, are exposed to the sun.

Although plowing, alone, will not eradicate nematodes from fields, it may be, nevertheless, a factor in their partial control in pineapple fields. Bessey (1), Godfrey (9), and Newhall (13), have discussed the depth distribution of this species of nematode in mainland soils. It is quite apparent from their investigations, that they are found at least 27 to 36 inches beneath the surface. But in these instances the tillable soil was very deep. Pineapple roots are largely, if not entirely, confined to the topsoil. Plowing depths in pineapple fields, at times include a little of the subsoil each time. Therefore, most of the soil inhabited by nematodes is treated by this cultural method. It would appear to be conclusive from this fact, that nematode control by plowing is an important field operation in pineapple culture.

Shindo (16) recently has shown that the greatest concentration of nematodes of all species in Japan is in the first 3 inches of soil. Unfortunately, the preferred soil depth of no particular species is indicated. Also, he found that nematode populations appear to be smallest during September in fallow land, perhaps because of lethal temperatures at higher levels, though this explanation is not suggested by him. Should these results prove to be true in the Hawaiian Islands too, disking as well as plowing fallow fields, especially in August and September, would be highly desirable in order to reduce further the existing populations of the root knot nematode, for each operation would serve to expose a very large proportion of them to lethal temperatures.

Mechanical transport of nematodes

Several writers have asserted that the root knot nematode may be carried from field to field by mechanical agencies such as the run-off of rain water and the feet of men and horses. None of them have discussed the particular conditions under which this might, or might not, be done. Not all are cited but examples of these general statements can be found in the papers by Bessey and Byars (2), Childs (5), Fuller (6), Gilbert (7), Godfrey (8), Rolfs (14), Schofield (15), Vosbury and Winston (21). Of course, this may be the case where more than the soil surface is transported, roots are carried, or soil from damp and shaded places is taken, but all of these instances are special cases. Without doubt such transference is common for *Heterodera schachtii* Schmidt when soil from sugar beets at the dump or mill is returned to the beet fields by the wagon load (18, 20). But it seems safe to conclude, from the foregoing data, that ordinarily one may walk through Hawaiian pineapple fields, and horses and cultivators may pass from one fallow field to another, without danger of spreading the root knot nematode, provided fresh soil from levels below 1 or 2 inches depth, or weed roots, are not carried at the same time. It appears highly improbable that winds, especially the light whirlwinds that occur so commonly both on the mainland United States and in the Hawaiian Islands, are carriers of living nematodes. Even should it be done, it would be difficult to conceive how the widely distributed larvae and eggs could survive long enough to reach safe levels in the earth when spread thinly over the surrounding country. This matter is even more to the point when applied to mainland conditions, where extremes of summer maximum air temperatures perhaps are greater than in the Hawaiian Islands.

In bringing this discussion to a close it should be stated that there has been no disposition to insist that no nematodes are to be found at the surface at depths of 1 to 3 inches. It has simply been pointed out that, at times, lethal temperatures for the root knot nematode actually do exist in the upper soil layers.

SUMMARY AND CONCLUSIONS

Air and soil temperatures during relatively dry and warm periods were taken at the Wahiawa field experiment station of the Association of Hawaiian Pineapple Canners, in the heart of the pineapple section. Air temperatures at Wahiawa and at Schofield Barracks are practically the same.

Uncovered soils at ¼-inch depth frequently rise above 40°C. for 2 hours or more, except during the coldest parts of the year extending from December to the following early summer months of April or May. In July and October the soil at a depth of 3 inches occasionally has temperatures between 38 and 40°C. for 2 hours or more.

Soils covered with mulch paper average 7°C. higher in temperature than uncovered soil at ¼-inch depth, and at 3 inches the average increase in temperature is about 6.5°C. over the corresponding depth temperature for uncovered soil. At the latter depth, daily temperatures above 40°C. are frequently attained for more than 2 hours in summer, and occasionally in the later months of the year. Apparently mulch paper covered soils do not have temperatures lethal for nematodes at a depth of 4 inches.

Soil with a dead air space covered by cellophane paper shows a marked increase in temperature. Individual daily temperatures, even at a 3-inch depth, often exceed 40°C. for more than 2 hours. One peak temperature in summer reached 46°C. for a short time.

Air and all ¼-inch depths in soil, attained their maximum temperatures between 2 and 3 p.m., after which they declined at least through early evening. Temperatures increased at the 3-inch level until about 3 p.m., and continued at that level until after 4 p.m. before decline was evident.

There seems to be no relationship between temperature and the velocity of the northeast trade winds.

Since a temperature of 40°C. for 2 hours and 15 minutes is lethal to the larvae of *Heterodera radicicola*, it is quite apparent that this nematode cannot survive at ¼-inch depth in the soil during the greater part of the year, when the soil is not shaded by vegetation. At a depth of 3 inches during the summer months, and perhaps until November, at least, the temperature of exposed soil approaches this critical point at intervals so that comparatively few, if any, nematodes find a suitable environment above this level at any time in fallow fields. Lethal temperatures for larvae in the soil under mulch paper are attained frequently a depth of about 3 inches.

From the standpoint of nematode control, summer plowing is decidedly beneficial since it brings a portion of the nematode population at lower levels within the influence of high temperatures. Disking fallow fields also should prove of assistance in exposing to lethal temperatures nematode populations which are at about the 3-inch level in the soil.

Nematode distribution from infested fields into uninfested areas by such mechanical means as transport on shoes, the feet of horses, and winds seems to be highly improbable when only the first inch or two of soil is carried away, especially under temperature conditions existing in the Hawaiian Islands.

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AN OBSERVATION ON THE BEHAVIOR OF SOIL UNDER REFORESTATION

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"Wheat was ripe here in 1842" reads a sign along the Black River road north of Bessemer in Gogebic County, Michigan. One reads this sign with much skepticism, for at the time he is passing through an area still covered with the primaeval forest. Community report supports the statement, but considerable doubt regarding the authenticity of the claim has been expressed by some of the local residents. To obtain indisputable evidence as to whether or not the land had been under cultivation at some time in the past, the soil was examined. A very thin accumulation of litter was found but under this virtually no "mold" or F layer. The underlying soil was rich in humus to a depth of approximately 7 inches and possessed a granular structure typical of the plowed layer in loam soils of medium to high organic content. An examination of the soil a few rods away in the virgin forest showed a normal accumulation of litter with "mold" or F layer about 2 inches thick. The humus soil below the "mold" was approximately an inch thick and was underlain by a grayish podzolized horizon. A comparison of the A horizons of the two soils left no doubt that the area in question had been under cultivation. An examination of the Land Office records substantiated this conclusion.

From a forester's standpoint the area is of much interest since some of the timber is of considerable size. Measurements of a number of the larger trees gave the following data:

	BASSWOOD	HEMLOCK	YELLOW BIRCH	HARD MAPLE	ELM	ASH	ASPEN
Circumference (inches)	49 49	58	32 42	34 40	45	40	44

The exact date at which this land was abandoned for farming and permitted to reseed to forest trees has not been ascertained. Examinations by Professor R. H. Westveld of the forestry department, Michigan State College, however, showed a large majority of the trees to be of a uniform age of 70 years. This observation indicates a common date of seeding and suggests that difference in sizes of trees are due largely to difference in opportunity for rapid growth caused by competition. The general height of the timber is only about 10 feet less than the height of the surrounding virgin timber.

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[1] Professor of soils, Published with permission of the director as Journal Article 126 (n.s.)

From a soils standpoint the observation is of interest since the failure to develop an appreciable layer of "mold" during the period required for the growth of trees of the sizes mentioned, gives some comprehension of the time required for the accumulation of "mold" of the thickness usually found in areas bearing similar forest cover.

The fact that the soil has retained a structure similar to that of plow soil and that during the period of reforestation no evidence of podzolization has appeared is also worthy of note since it indicates that modification of soil characteristics as a result of forest growth is a very slow process.

PLATE 1

SECTIONS OF SURFACE SOIL (RIGHT) FROM VIRGIN TIMBER AND (LEFT) FROM AN AREA WHICH WAS CULTIVATED PREVIOUSLY BUT ALLOWED TO REVERT TO FOREST APPROXIMATELY 70 YEARS AGO

Note absence of "F" horizon or mold, the granular structure, and the absence of a podzolized layer in the soil previously under cultivation. Observe in the specimen from virgin timber the well-defined podzolized zone with no evidence of granulation, and that the mold rests almost directly on the podzol with a very thin layer of humus soil.

A COMPARATIVE STUDY OF CROPPED AND VIRGIN SOILS[1]

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Several workers have reported studies in which partial analyses were made of cropped and the corresponding virgin soils. Most of these included the determination of one or more of the following: pH, phosphorus, nitrogen, calcium, magnesium, composition of soil solution, and exchangeable calcium. A number of workers have reported results of base exchange studies on soils that have been cropped for different periods of time. In general, these studies deal with soils which have received constant fertilizer treatment for a great many years. In some cases, however, unfertilized soils have been included.

In so far as the writer is aware, little information is available regarding the exchangeable bases and composition of cropped soils and of the corresponding adjacent virgin soils. A study of exchangeable bases together with a chemical analysis of cropped and virgin soil should throw some light on the changes which a soil has undergone by continuous cropping. The relation between the change in the composition of the absorption complex in the soil, due to cropping, and the chemical composition of the soil has not been established. The change in the relative proportion of the bases in the soil with the change in the total of the bases is uninvestigated. The removal of bases from the absorption complex by cropping must be followed by a continuous process of recovery or we would have after a relatively short period of cropping a complex with practically all bases removed. The proportions of the different bases in the soil that are exchangeable should give some indication of the capacity of the soil to furnish plant nutrients. It is the purpose of this paper to throw some light on these questions and to set forth any relationships found to exist between the amounts of various plant food elements in cropped and the corresponding adjacent virgin soils and the exchangeable bases.

LITERATURE CITED

The literature of the subject base exchange has been extensively reviewed in a number of recent publications, therefore an exhaustive review is unnecessary in this paper. Many papers have been called to the attention of the writer, but only those dealing more or less specifically with the subject of the paper will be cited.

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[1] A thesis presented to the faculty of Michigan State College of Agriculture and applied science in partial fulfillment of the requirements for the degree of doctor of philosophy. Published with permission of the director as journal article 130 n.s. of the experiment station.

[2] The writer wishes to express his sincere gratitude to Dr. C. E. Miller for helpful advice and criticism during the progress of the work and in the preparation of the manuscript.

Millar (12) determined the rate of formation of soluble material in several virgin and the corresponding depleted soils by means of the freezing point method. On the whole, the data showed that the surface soils of the virgin samples possessed the power of giving up soluble material at a greater rate than the corresponding cropped samples. No appreciable difference in the rate of solubility of the subsoils from the depleted and virgin soils was observed.

Martin (11), in studying soils in iron containers, cropped one set of soils to barley continually for 12 years. Another set was cropped to barley 1 year, fallowed 10 years, and again cropped to barley. Exchangeable bases were determined on these soils and also on samples of the original soils. No significant differences were noted in the exchangeable calcium, magnesium, and sodium of the continually cropped and fallowed soils; but exchangeable potassium showed a marked decrease as a result of cropping. This experiment was conducted under conditions which precluded the loss of any of the four bases from the soil except by crop removal.

Fraps (5) found that the quantities of water-soluble potassium, the potassium soluble in 12 per cent HCl, and the replaceable potassium in certain soils were all related to the potassium removed by crops in pot experiments and to the "active" potassium of the soils. The amount of potash taken up by two crops usually averaged five or six times the quantity of water-soluble potassium and about one-half the replaceable potassium in the soils.

Ogg and Dow (13) found decidedly more exchangeable calcium, a higher pH value, and lower "lime requirement" in cultivated soils as a group than in "woodland," "hill," or "heath" soils, or soils left "long unploughed." Almost double the quantity of exchangeable calcium was found in the surface 9 inches of one of the cultivated soils than in the adjoining uncultivated soil.

Gedroiz (6) made a study of the relation between exchangeable cations of soils and plant growth. He found that after the practically complete replacement from the soil of exchangeable calcium, the plants required for their development in this soil the addition of calcium, without which they did not grow at all. They were evidently unable to utilize the unexchangeable calcium of the soil. After the practically complete replacement from the soil of magnesium and potassium, plants were able to develop and give a more or less normal yield even without the introduction of magnesium and potassium fertilizers, showing that the plants were able to utilize the unexchangeable magnesium and potassium of the soil.

Ames and Schollenberger (1) determined the total calcium and magnesium and the calcium and magnesium soluble in 0.2 *N* nitric acid in several cropped and virgin soils of the same type. They found, in most instances, that the proportion of total calcium and magnesium, especially calcium, soluble in 0.2 *N* nitric acid was greater in the surface (0 to 7 inches) of virgin soil than in the same depth of cultivated soil. However, when the data for the 7- to 15-inch depth of these soils were compared, the reverse of this observation was found in many cases. In the total analyses for calcium and magnesium no consistent differences were shown.

Schollenberger and Dreibilbis (18), working with the soils of the 5-year rotation fertility plots at Wooster, studied the effect of cultivation with and without fertilizer and lime treatments upon the exchangeable bases of the soil. They found that the amounts of exchangeable manganese, calcium, magnesium, potassium, and sodium had decreased, whereas the exchangeable hydrogen had increased, as a result of 31 years of cropping without fertilizer or lime. There had been also a net decrease in the total bases and the exchange capacity.

Page and Williams (14) studied the content of exchangeable bases in the soil of certain plats on Broadbalk Field and the Grass Plats of Rothamsted. This work showed that the relative proportions of the different bases varied consistently with the manuring and that there was probably a gradual conversion of exchangeable potassium to a non-exchangeable form, or vice versa, depending on whether or not potassium fertilizers were used.

Crowther and Basu (4) studied the influence of fertilizers and lime on the replaceable bases in a light-textured, acid soil after 50 years of continual cropping with barley and wheat.

The replaceable calcium had decreased under all treatments. A mineral treatment increased the replaceable magnesium and potassium but it was insufficient to balance the loss of calcium.

Wilson (25), on making a study of exchangeable calcium and potassium in soils as affected by cropping and fertilization, observed that the exchangeable potassium and, with one exception, the exchangeable calcium in the untreated soils during the 15 years of the experiment, were reduced. These soils were held in cylinders but were subject to leaching.

Hissink (8) states that the non-exchangeable potassium of soils changes to exchangeable potassium.

SOILS USED

The soils investigated are classified as Plainfield loamy sand, Coloma loamy sand, Hillsdale sandy loam, "College Farm" (Hillsdale sandy loam), Miami loam, Nappanee silt loam, and Ontonagon clay loam. Soil survey reports (20, 21, 22) give descriptions of these soil types.

TREATMENT OF CROPPED SOILS

The Coloma, Hillsdale, "College Farm," and Plainfield soils were obtained in Ingham County. They are well-drained soils and were among the first soil types to be cleared and farmed in the county. These soils have been cultivated. for more than 60 years in what might be called general or mixed type of farming The number of livestock kept was not large, and corn, oats, wheat, rye, and mixed hay were the crops usually grown. For a considerable number of years clover has failed, and hence the hay was largely timothy. The Coloma soil has grown nothing but corn for a number of years. The field was not planted every year.

The samples were taken from fields located a considerable distance from the barns and have received only an occasional application of manure. No lime or commercial fertilizer has been applied to these soils.

The Miami soil is also from Ingham county, and has been under cultivation from 40 to 50 years. The system of farming was much better than that practiced on the average farm. General farming has been followed with the most common crops—corn, oats, wheat, barley, and clover. This soil has received liberal amounts of barnyard manure.

The Nappanee soil is from Allegan County and has been cultivated about 60 years. This soil has been under general farming practices and has not received any commercial fertilizer or lime treatments. It has had a number of light applications of barnyard manure.

The Ontonagon soil is from Chippewa County and has been under cultivation about 20 years. This soil has been in timothy, and alsike clover for hay, most of the time, with a grain crop when it became necessary to plow the soil to kill the weeds. No lime, fertilizer, or manure has been applied to this soil.

COLLECTION OF SAMPLES

The samples of cropped soil represent the surface stratum of 6 or more inches depending upon the depth to which the soil had been plowed. The virgin soils were sampled to what was considered a corresponding depth the thickness of the F horizon being taken into consideration. Careful attention in the selection of fields for sampling was necessary in order to select cultivated fields that apparently were free from erosion and had received no lime or commercial fertilizer. A desire to take the virgin soil sample from a tract of woodland only a short distance from the site of the first sample made this task more difficult. No soil samples taken from fence rows are included in this investigation, for these soils represent a more or less prairie condition. In taking these samples the litter of the virgin soils was not included, as in clearing land for cultivation this material is largely destroyed.

Two samples were taken from each soil type: one from a field which had been under cultivation for a considerable number of years and had decreased more or less in productivity; and the other from a virgin woodland soil a short distance from the site of the first sample. The first sample is designated as cropped soil, and the latter is referred to as the virgin soil.

The samples were taken to the laboratory, air dried, and passed through a 2-mm. mesh sieve to remove pebbles and the coarser organic material. They were then thoroughly mixed and stored in suitable containers until utilized in the experiments.

METHODS

The methods used in these determinations may be divided into two groups: first, methods dealing with chemical analysis of the soil, and second, those concerned with some phase of base exchange. In this paper, however, no detailed procedures will be given.

In the total analysis of the soil, the methods as outlined by the Association of Official Agricultural Chemists (2) were used for determining total nitrogen and phosphorus. Their methods were also used for making the sodiumc arbonate fusion and the determinations of silica, iron, aluminum, and calcium. Magnesium was determined by Handy's (7) well-known method. Sodium and potassium were determined by the procedure described by Scott (19). Hydrogen-ion concentration was measured by the use of the quinhydrone electrode as suggested by Baver (3).

Schollenberger (16) points out the advantageous features of a neutral solution of ammonium acetate for the estimation of all exchangeable cations in the soil. The methods and apparatus described by Schollenberger and Driebilbis (17) were used in the extraction and determination of the exchangeable cations with the exception of exchangeable ammonium, which was determined by the method of McLean and Robinson (10). The procedure of Kelly and Brown (9) slightly modified by Schollenberger and Driebilbis (17) was followed in the determination of absorbed ammonium. The available or easily replaced, exchangeable calcium was estimated by the procedure of Parker and Pate (15). In determining readily available phosphorus the method outlined by Truog (24) was employed.

CHEMICAL ANALYSIS OF THE SOIL

It has been stated that chemical composition is closely related to the wearing qualities of the soil and its ability to persist in production under cultivation. One of the objects of this study was to learn the effect of cropping on the chemical composition of soils. The analyses are shown in table 1.

With the exception of nitrogen content, a comparison of analyses of cultivated and virgin samples of the seven soil types, as a group, shows no significant differences. However, when the data are studied in regard to any given soil type, in many instances wide differences are noted. These differences within any given type are usually reversed in one of the other types. Of the soil types studied, there is no significant difference in the silica content of cultivated and virgin soil. In the iron content a difference greater than the experimental error is found in three soil types. In the College Farm (Hillsdale sandy loam) and Miami loam the virgin soil contains more iron than the cultivated. This order is reversed in the case of Nappanee silt loam. The Nappanee silt loam shows a higher aluminum content in the cultivated state than in the virgin condition. The amount of aluminum in cultivated and virgin Coloma loamy sand is the same, but the other types have slightly more in the virgin soil.

TABLE 1

Analyses of cropped and the corresponding virgin soils

SOIL DESCRIPTION	SiO_2	Fe_2O_3	Al_2O_3	CaO	MgO	K_2O	Na_2O	N	P_2O_5
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Hillsdale sandy loam:									
Cultivated	83.80	2.09	6.81	0.65	0.36	1.68	2.40	0.102	0.101
Virgin	85.15	2.04	7.12	0.68	0.37	1.55	2.28	0.107	0.116
College farm:									
Cultivated	84.07	1.95	6.61	0.63	0.36	1.87	2.80	0.099	0.094
Virgin	82.74	2.23	7.05	0.65	0.38	1.63	1.94	0.111	0.100
Miami loam:									
Cultivated	79.01	2.15	7.48	0.94	0.55	2.21	2.32	0.209	0.156
Virgin	81.00	2.34	7.73	0.80	0.46	2.09	3.78	0.127	0.095
Coloma loamy sand:									
Cultivated	88.95	1.47	4.90	0.61	0.28	1.61	2.74	0.081	0.116
Virgin	87.81	1.39	5.01	0.61	0.25	1.65	2.64	0.091	0.116
Plainfield loamy sand:									
Cultivated	87.34	1.86	5.60	0.67	0.33	1.66	2.46	0.054	0.108
Virgin	85.75	1.76	5.60	0.70	0.32	2.25	2.98	0.064	0.139
Ontonagon clay:									
Cultivated	67.30	5.43	13.80	1.03	1.19	3.24	3.60	0.176	0.109
Virgin	66.94	5.47	15.00	1.03	0.92	3.68	4.06	0.180	0.090
Nappanee silt loam:									
Cultivated	79.64	2.99	8.51	0.36	0.61	1.87	2.40	0.139	0.103
Virgin	80.10	2.33	7.25	0.43	0.44	2.19	2.56	0.171	0.090

No consistent differences were found in the calcium and magnesium content of the virgin and cultivated soil samples of the soil types studied. This result is in agreement with the findings of Ames and Schollenberger (1) in their determination of total calcium and magnesium. The calcium and magnesium content of cultivated Miami loam is considerably greater than that of virgin Miami loam. A difference in the same direction is found in the Ontonagon clay and Nappanee silt loam. The percentage of MgO is greater than the percentage of CaO in cultivated Ontonagon clay and in both soils of Nappanee silt loam. However, when these percentages are calculated to per cent calcium and magnesium, the per cent of magnesium is greater only in the cultivated Nappanee soil.

As in the case of calcium and magnesium there is no consistent difference in the potassium and sodium contents of the virgin and cultivated soils. When each type is considered separately we find some marked differences. Four of the soils contain more potassium in the virgin samples and three contain more in the cultivated. Some of these differences are great enough to be significant. Since all the error in determining sodium and potassium falls on the sodium determination, in order to be of value appreciable difference in the sodium content of the samples is necessary. The cultivated soil of the College Farm, the virgin soil of Miami loam, and the virgin Plainfield soil contained noticeably more sodium than the corresponding virgin or cultivated soils, respectively, of these types.

When a comparison is made of the nitrogen contents on the percentage basis it is found that with one exception there has been a decrease in the nitrogen content due to cropping. This is in agreement with the findings of a number of investigators working with a large variety of soils. Some of these results have been reviewed by Swanson and Latshaw (24). With the exception of one type, the cultivated soil contains from 100 to 440 pounds less nitrogen per acre than the virgin soil. This means a loss of 5 to 20 per cent of the original amount of nitrogen. The cultivated Miami loam contains 1,640 pounds more nitrogen per acre than the virgin soil. This great increase is undoubtedly due to the system of farming.

In comparing the amounts of phosphorus found in these soils there is no difference consistent throughout the types. However, if the soils are divided into the lighter and heavier soils good agreement is found. The phosphorus content of the virgin soil is used as a basis of comparison. In the four lighter soils the virgin soil contains from 0 to 600 pounds more phosphoric acid per acre than the cultivated soils. This means a loss of 0 to 20 per cent of the original amount of phosphoric acid. In the three heavier soils the cropped soil contains from 140 to 1,220 pounds more phosphoric acid than the virgin soils. This means a gain of 7.5 to 64 per cent of the original amount of phosphoric acid. The gain of 64 per cent was made by the Miami loam which made such an increase in nitrogen content. This increase in the heavier soils is possibly due to a greater growth of crops and the consequent bringing up of more phosphorus from the lower horizons.

EXCHANGEABLE BASES

The analytical date for the base exchange studies are presented in table 2, together with the milliequivalents of ammonium ion absorbed and the pH values.

In practically all cases little difference is found in exchangeable hydrogen between cropped and virgin soils of the same soil type. The pH values of these soils are noticeable because of the very close agreement between the results from the cropped and virgin samples. These data show that there has been no change in pH due to cropping, and no correlation between exchangeable hydrogen and pH is shown; however, there is a correlation between texture and replaceable hydrogen. This is noted particularly in Hillsdale sandy loam—pH 5.40, Nappanee silt loam—pH 5.41, and Ontonagon clay—pH 5.41. The exchangeable hydrogen in these soils is 1.72, 2.89, and 5.03 m.e., respectively.

TABLE 2

Exchangeable bases, absorption capacity, and pH of cropped and virgin soils

Expressed as milligram-equivalents per 100 gm. air-dry soil

SOIL DESCRIPTION	EXCHANGEABLE BASES										
	H	Al	Mn	Ca	Mg	K	Na	NH_4	Base total	NH_4 absorbed	pH
Hillsdale sandy loam:											
Cultivated	1.32	0.11	0.14	3.86	0.44	0.08	0.30	0.06	6.31	6.37	5.36
Virgin	0.88	0.13	0.11	3.49	0.38	0.08	0.22	0.11	5.39	5.29	5.36
College farm:											
Cultivated	0.74	0.11	0.19	3.39	0.45	0.09	0.22	0.01	5.19	5.07	5.40
Virgin	1.72	0.10	0.14	4.15	0.89	0.19	0.25	0.07	6.51	6.81	5.40
Miami loam:											
Cultivated	0.90	0.04	0.11	10.61	2.47	0.12	0.24	0.06	15.63	14.15	6.27
Virgin	1.20	0.05	0.12	8.14	1.07	0.14	0.22	0.02	10.96	9.86	6.18
Coloma loamy sand:											
Cultivated	0.30	0.03	0.07	4.41	0.84	0.19	0.16	0.00	6.00	4.72	6.01
Virgin	0.30	0.04	0.08	5.76	1.22	0.14	0.21	0.00	7.45	5.27	6.04
Plainfield loamy sand:											
Cultivated	0.92	0.08	0.11	1.79	0.21	0.04	0.18	0.09	3.42	3.86	5.40
Virgin	1.40	0.07	0.12	0.84	0.22	0.06	0.24	0.09	3.04	3.69	5.48
Ontonagon clay:											
Cultivated	5.03	0.23	0.37	9.41	4.05	0.40	0.45	0.07	20.01	19.71	5.41
Virgin	4.78	0.21	0.34	9.47	2.21	0.44	0.35	0.16	18.00	17.76	5.48
Nappanee silt loam:											
Cultivated	2.89	0.17	0.27	4.70	1.02	0.22	0.33	0.17	9.77	9.77	5.45
Virgin	2.80	0.19	0.28	4.48	1.04	0.19	0.22	0.13	9.33	9.44	5.41

The content of exchangeable aluminum and manganese in the soils studied was relatively low with no consistent increase or decrease due to cropping. The amount of exchangeable aluminum and manganese had a positive correlation with the exchangeable hydrogen. This is in agreement with the findings of many workers.

The quantities of exchangeable bases calcium, magnesium, sodium, and potassium vary widely when each type is considered. The greatest variations in calcium are found in the lighter soils. Two soils, College Farm and Coloma loamy sand, indicate that there has been a considerable decrease in exchangeable calcium, whereas three soils indicate an increase.

As judged by the mean values for the group, magnesium shows a slight increase in the cropped soils. However, two soils show a very marked decrease in magnesium. These two soils, Coloma loamy sand and College Farm, also show a marked decrease in exchangeable calcium. Sodium does not seem to have undergone any uniform or significant change, the average content for all the soil types being nearly constant. There are about an equal number of increases and decreases in sodium content of the cropped soils, using the virgin soils as standards, with a slight increase indicated.

The content of exchangeable potassium does not seem to have undergone any large change. Outside of the loss of more than 50 per cent of the exchangeable potassium in the cropped College Farm soil there was no other large difference found as a result of cropping. Four soils indicate a decrease and two an increase in exchangeable potassium. From the small quantity of exchangeable potassium found in these soils, both cropped and virgin, it is evident that crops on these soils obtain their potassium from some source in addition to the exchangeable potassium in the surface soil, undoubtedly in part from the exchangeable potassium in the subsoil and probably from potassium that is in a non-exchangeable form.

The total bases found and the saturation capacity show good agreement except in the case of Coloma loamy sand where the total bases are considerably greater than the absorption capacity. As in the cases of the individual bases there is no consistent difference throughout the types. Five soils indicate an increase in base capacity and total bases in the cropped soils, and two indicate a decrease. The increase of the absorption capacity in the cropped soil, using the virgin soil as a standard of comparison, varies from 3.5 per cent in the Nappanee silt loam to 43 per cent in Miami loam. The decreases in absorption capacity are shown by Coloma loamy sand and Hillsdale sandy loam. The high percentage of increase shown by Miami loam is undoubtedly in part due to an increase in the organic exchange complex, as the cropped soil shows a marked increase in organic matter.

TOTAL BASES AND EXCHANGEABLE BASES

The relationship that exists between the total bases in the soil and the amount of exchangeable bases should be of interest. The relative proportions of the different bases that are in the exchangeable form should give some indication of the capacity of the soil to furnish these bases. The percentage of the total of each base that is exchangeable should give a good indication of the changes that have occurred in the soils studied. In table 3 the percentage of bases and exchangeable bases and percentage of total bases exchangeable are given. These data are on the elements in all cases; that is, percentage of calcium, and milliequivalents, exchangeable calcium calculated to percentage of exchangeable calcium.

TABLE 3

Percentage bases and exchangeable bases and percentage of total bases exchangeable in cropped and virgin soils

SOIL DESCRIPTION	CALCIUM			MAGNESIUM			POTASSIUM			SODIUM		
	Total	Exchangeable	Percentage of total exchangeable	Total	Exchangeable	Percentage of total exchangeable	Total	Exchangeable	Percentage of total exchangeable	Total	Exchangeable	Percentage of total exchangeable
	per cent	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*	
Hillsdale sandy loam:												
Cultivated.......	0.46	0.077	16.60	0.22	0.0050	2.47	1.35	0.0031	0.23	1.78	0.0069	0.39
Virgin...........	0.49	0.070	14.36	0.22	0.0046	2.07	1.24	0.0031	0.24	1.69	0.0051	0.30
College farm:												
Cultivated.......	0.45	0.070	15.06	0.22	0.0055	2.52	1.50	0.0035	0.23	2.08	0.0051	0.24
Virgin...........	0.46	0.083	17.88	0.23	0.0108	4.72	1.31	0.0074	0.55	1.44	0.0058	0.30
Miami loam:												
Cultivated.......	0.67	0.212	31.60	0.33	0.0300	9.05	1.77	0.0047	0.25	1.72	0.0055	0.40
Virgin...........	0.57	0.163	26.45	0.28	0.0130	4.69	1.68	0.0055	0.31	2.80	0.0053	0.19
Coloma loamy sand:												
Cultivated.......	0.44	0.088	20.24	0.17	0.0102	6.05	1.29	0.0074	0.55	2.03	0.0037	0.18
Virgin...........	0.44	0.115	36.96	0.15	0.0148	9.83	1.32	0.0055	0.38	1.96	0.0048	0.24
Plainfield loamy sand:												
Cultivated.......	0.48	0.036	7.48	0.20	0.0026	1.28	1.33	0.0016	0.11	1.83	0.0041	0.23
Virgin...........	0.50	0.017	3.36	0.19	0.0027	1.39	1.81	0.0023	0.12	2.21	0.0055	0.26
Ontonagon clay:												
Cultivated.......	0.74	0.188	25.58	0.72	0.0492	6.84	2.60	0.0156	0.58	2.67	0.0104	0.39
Virgin...........	0.74	0.189	25.75	0.55	0.0269	4.84	2.96	0.0172	0.57	3.01	0.0081	0.27
Nappanee silt loam:												
Cultivated.......	0.26	0.094	36.55	0.37	0.0124	3.36	1.50	0.0086	0.55	1.78	0.0076	0.43
Virgin...........	0.31	0.090	29.23	0.27	0.0126	4.76	1.76	0.0074	0.41	1.90	0.0051	0.27

The mean values show the percentage of the total calcium that is exchangeable to be almost constant. Four soils show a greater percentage of the total calcium in an exchangeable form in the cropped soils, and three cropped soils show a smaller percentage to be exchangable than in the corresponding virgin soils. When the different types are considered as a unit very little correlation is indicated between the quantities of total calcium and exchangeable calcium.

The percentage of calcium that is in the exchangeable form increases as the soil becomes heavier, with the amount exchangeable ranging from 3.36 per cent in virgin Plainfield loamy sand to 36.55 per cent in cultivated Nappanee silt loam.

The percentage of the total magnesium exchangeable varies considerably in different soil types. Some cropped soils show a marked decrease while others show a large increase over the corresponding virgin soils. In most cases, these changes in any given type are in the same direction as the changes in the percentage of total calcium exchangeable. The average for the cropped soils is 4.51 per cent, and the average for the virgin soils is 4.61 per cent; thus the mean values indicate that the percentage of total magnesium exchangeable is about constant. The relative proportion of magnesium exchangeable increases as the soils become heavier.

The data indicate no correlation between the total potassium and the amount exchangeable. The percentage of the exchangeable potassium increases as the soil becomes heavier. The College Farm soil shows the greatest decrease, more than 50 per cent, in the percentage of the total potassium exchangeable. There are about an equal number of increases and decreases with the mean values for both cropped and virgin soils indicating very little change in the percentage of the total potassium that is exchangeable. The percentage of the total potassium that is exchangeable ranges from 0.1 per cent in cropped Plainfield loamy sand to 0.58 per cent in cropped Ontonagon clay.

The mean values for the percentage of the total sodium that is exchangeable show a 14.8 per cent increase in the cropped soil. Since the error of the determination of sodium and potassium falls on the sodium determination, this increase is not considered very significant. There are about an equal number of increases and decreases in the cropped soils.

The data in table 2 show very little change in the saturation compacity of these soils after continued cultivation. Wide variations have been noted in certain types, but little difference was found in others.

Since the mean values for the percentages of the bases that are exchangeable are nearly constant, it seems that there may be an equilibrium between the bases in the exchangeable form and those in the non-exchangeable form. This would involve a continuous exchange between the two forms of bases. This change is probably very slow.

The percentage of the total bases exchangeable increases as the soil becomes heavier. This is to be expected when we consider that the sandy soils are very low in colloidal material.

The mean values for the percentages of the total bases exchangeable in both cropped and virgin soils are as follows: calcium, 21.92; magnesium, 4.56; potassium, 0.365; and sodium, 0.29 per cent. This means the average of these soils contains enough calcium to replace the present amount exchangeable 4.5 times; the magnesium is capable of 22 replacements; potassium, 275; and the sodium, 345 replacements.

PERCENTAGES OF THE TOTAL BASES AND OF TOTAL BASES EXCHANGEABLE COMPRISED OF DIFFERENT BASES

The relative proportions of the bases calcium, magnesium, potassium, and sodium constituting the total of these bases and exchangeable cations in the soils studied are given in table 4. That is, calcium constitutes 12.1 per cent of the total bases and 82.5 per cent of the exchangeable bases of cultivated Hillsdale sandy loam. Sodium constitutes the highest percentage of the total bases, followed by potassium, calcium, and magnesium, in the order named.

TABLE 4

Percentages of the total bases and of the total exchangeable bases constituted by different bases

SOIL DESCRIPTION	TOTAL BASES				EXCHANGEABLE BASES			
	Calcium	Magnesium	Potassium	Sodium	Calcium	Magnesium	Potassium	Sodium
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Hillsdale sandy loam:								
Cultivated	12.1	5.8	35.4	46.7	82.5	9.4	1.7	6.4
Virgin	13.5	6.0	34.1	46.4	83.7	9.1	1.9	5.3
College farm:								
Cultivated	10.6	5.2	35.3	48.9	81.7	10.8	2.2	5.3
Virgin	13.4	6.7	38.1	41.9	75.7	16.2	3.5	4.6
Miami loam:								
Cultivated	14.9	7.3	39.4	38.3	78.9	18.4	0.9	1.8
Virgin	10.7	5.3	31.5	52.5	85.1	11.2	1.5	2.3
Coloma loamy sand:								
Cultivated	11.2	4.3	32.8	51.7	78.8	15.2	3.4	2.9
Virgin	11.4	3.9	34.1	50.6	78.6	16.6	1.9	2.9
Plainfield loamy sand:								
Cultivated	12.5	5.2	34.6	47.7	80.6	9.5	1.8	8.1
Virgin	10.6	4.0	38.4	46.9	61.8	16.2	4.4	17.6
Ontonagon clay:								
Cultivated	11.0	10.7	38.6	39.7	65.8	28.3	2.8	3.1
Virgin	10.2	7.6	40.8	41.5	75.9	17.7	3.5	2.8
Nappanee silt loam:								
Cultivated	6.6	9.5	38.4	45.5	75.0	16.3	3.5	5.3
Virgin	8.5	6.3	41.5	44.8	75.5	17.5	3.2	3.7

Calcium is predominant in the exchangeable cations followed by magnesium sodium, and potassium respectively. The ratio between the exchangeable cations is in good agreement with the findings of other investigators.

In general, as the soils become heavier the relative proportion of the total bases and exchangeable cations constituted by calcium, decreases. The reverse of this is true for magnesium and potassium, whereas sodium shows little or no difference.

The difference between cropped and virgin soils shown by the data in table 4 have already been discussed.

AVAILABLE PHOSPHORUS, PERCENTAGE OF TOTAL PHOSPHORUS AVAILABLE, AND AVAILABILITY OF EXCHANGEABLE CALCIUM

The question may well be asked, does the availability of the nutrient elements in these soils differ? In order to obtain some information on this question the available phosphorus was determined by Truog's method, and the readily available exchangeable calcium by the method of Parker and Pate.

TABLE 5

Total phosphorus, readily available phosphorus, and percentage of total phosphorus readily available, and exchangeable calcium, readily exchangeable calcium, and percentage of total calcium readily exchangeable

SOIL DESCRIPTION	PHOSPHORUS			CALCIUM		
	Total	Readily available	Percentage of total readily available	Exchangeable	Readily exchangeable	Percent of total readily exchangeable
	p.p.m.	*p.p.m.*		*m.e.*	*m.e.*	
Hillsdale sandy loam:						
Cultivated	440	16	3.63	3.89	0 51	13.21
Virgin	508	20	3.93	3.49	0.47	11.41
College farm sandy loam:						
Cultivated	410	14	3.33	3.39	0.46	13.57
Virgin	436	17	3.85	4.15	0.43	10.36
Miami loam:						
Cultivated	680	18	2.69	10.61	1.87	17.62
Virgin	414	13	3.23	8.14	1.29	15.87
Coloma loamy sand:						
Cultivated	508	62	12.30	4.41	0.55	12.47
Virgin	508	64	12.70	5.76	0.79	11.98
Plainfield loamy sand:						
Cultivated	472	19	4.12	1.79	0.21	11.73
Virgin	606	44	7.30	0.84	0.095	11.28
Ontonagon clay:						
Cultivated	472	20	4.19	9.41	1.61	17.11
Virgin	392	15	3.91	9.47	1.35	14.26
Nappanee silt loam:						
Cultivated	450	14	3.10	4.70	0.63	13.40
Virgin	392	14	3.44	4.48	0.54	12.05

The results, together with the total phosphorus and total exchangeable calcium of the soils, are given in table 5. The phosphorus is expressed in parts per million, and the percentage of the total phosphorus that is readily available is calculated. The calcium is expressed in milliequivalents. The percentage of readily available phosphorus as shown in table 5 includes some of the difficultly available phosphorus.

The amount of available phosphorus is rather low when all soil types are considered, ranging from 13 p.p.m. in virgin Miami loam to 64 p.p.m. in virgin

Coloma loamy sand. The per cent of the total phosphorus available ranges from 2.69 in cultivated Miami loam to 12.7 in virgin Coloma loamy sand. A factor which probably causes a low amount of available phosphorus to be present in these soils is the high hydrogen-ion concentration. With the exception of cultivated and virgin Coloma loamy sand and virgin Plainfield loamy, sand, the quantity of readily available phosphorus in these soils would be considered below the minimum limit for general farming.

The amount of readily available phosphorus follows the total phosphorus content to a fair degree in these soils. With one exception, Ontonagon clay, the percentage of the total phosphorus readily available is greater in the virgin soil of each type.

It is probable that the availability of the exchangeable bases in a soil is important in the nutrition of plants. The case of replacement from the absorbing complex of the soil might be related to the plant's response to these bases. The method of Parker and Pate (15) for estimating the availability of exchangeable calcium is based on this principle. In this method equal quantities of exchangeable calcium and potassium in potassium acetate compete for the absorbing complex of the soil.

The exchangeable calcium found readily available by this method ranges from 10.36 per cent in the virgin College Farm soil to 17.62 per cent in the cultivated Miami loam. In all types studied the percentage of exchangeable calcium readily available was found to be greater in the cultivated soil. Because the hydrogen-ion concentrations of these soils are grouped so closely it is impossible to determine whether there is any relationship between the hydrogen-ion concentration and readily available exchangeable calcium. The results obtained on these soils indicate that the exchangeable calcium is more closely held in the absorbing complex of the virgin soils.

CROP YIELDS OF CULTIVATED AND VIRGIN SOILS

In view of the small differences between the cultivated and corresponding virgin soils found in the chemical studies it was felt that the growth of the crops on these soils would be of interest and might indicate differences between the soils not detected by the chemical determinations. With this in view those soil types of which there was a sufficient quantity of the original samples were potted and seeded to barley. After the barley was harvested, the soil was screened to remove roots and repotted, then seeded to barley. The fertilizer treatments were not repeated for the Sudan crop.

Because of an insufficient amount of soil, only four sets of pots were possible for each soil. These pots were treated in duplicate as follows: (*a*) no treatment, (*b*) 400 pounds of 20 per cent superphosphate per acre, (*c*) 400 pounds of superphosphate, and 200 pounds of sodium nitrate per acre, (*d*) 400 pounds of 20 per cent superphosphate, 200 pounds of sodium nitrate, and 100 pounds of muriate of potash per acre. The results obtained are given in grams of air dry plant material in table 6.

When the data are considered as a group, the virgin soil gave a greater yield than the cultivated soil under similar conditions. In the Miami checks the cropped soil gave a greater yield of both barley and Sudan grass than did the virgin soil. In the checks of the other soil types the virgin soil gave increases with barley ranging from 4 per cent to 68 per cent and with Sudan grass ranging from 51 per cent to 364 per cent over the cropped soil. The average increase of the virgin checks over the cropped checks was 23 per cent for barley and 175 per cent for Sudan gras.

The pots which received 20 per cent superphosphate at the rate of 400 pounds to the acre show that neither the cropped nor virgin Coloma soil responds to phosphorus alone. This would be expected from the results found in laboratory determinations of readily available phosphorus by the Truog method which showed a sufficient amount of readily available phosphorus for plant growth in thse two soils. The results of the determination of readily available phosphorus is well supported by the greenhouse results.

TABLE 6

Yields of barley and Sudan grass, in grams of air-dry plant tissue, grown on differently treated cultivated and virgin soils in the greenhouse

SOIL DESCRIPTION	CHECK		PHOSPHORUS		NITROGEN PHOSPHORUS		NITROGEN PHOSPHORUS POTASSIUM	
	Barley	Sudan grass	Barley	Sudan grass	Barley	Sudan grass	Barley	Sudan grass
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Coloma:								
Cultivated	1.40	0.88	1.45	0.88	2.35	1.13	2.25	0.89
Virgin	2.35	1.23	2.40	0.99	2.58	1.09	3.07	1.11
College Farm:								
Cultivated	2.03	1.10	2.40	2.04	2.95	2.57	2.85	2.29
Virgin	2.12	3.55	2.93	3.28	3.00	4.23	3.33	5.76
Hillsdale:								
Cultivated	1.75	0.85	1.55	1.32	2.35	1.64	2.55	2.04
Virgin	1.75	2.92	2.35	3.17	2.75	3.58	2.70	2.69
Plainfield:								
Cultivated	0.95	0.50	0.95	0.57	1.80	0.63	2.00	0.69
Virgin	1.05	2.32	1.00	3.10	1.80	2.72	2.15	2.95
Miami:								
Cultivated	2.35	1.54	3.05	2.22	3.25	2.93	3.20	3.05
Virgin	2.15	1.29	3.35	2.65	3.60	3.02	3.70	2.58

The phosphorus treatment gave an average increase of 32 per cent over the checks. The virgin soils gave a greater response to phosphorus treatment than did the cropped soils with the barley, but the reverse was true with Sudan grass. In all cases phosphorus treated virgin soils gave more plant growth than the phosphorus treated cropped soils, the difference averaging 31 per cent for the barley and 138 per cent for the Sudan grass.

Both the cropped and virgin soils responded to nitrogen fertilization, the cropped soils giving a much greater response. Where 200 pounds of sodium nitrate was applied in addition to the 400 pounds of superphosphate per acre marked increase in growth was noted. The increased growth, for the two crops, amounted to an average of 35 per cent on the cropped soils over the corresponding soil receiving phosphate alone. The average yield on the virgin soil was 17 per cent greater for the nitrogen-phosphorus pots than for the phosphorus alone.

The greater plant growth on the cropped soil resulting from nitrogen fertilization is probably due to a much slower rate of decay of the organic matter of these soils, and therefore a less rapid liberation of available nitrogen. This explanation is supported by the fact that in some cases little difference in the total nitrogen content is found. The greater response of the cropped soils to nitrogen is shown again when the checks are compared with the nitrogen-phosphorus treatment. The cropped soils receiving nitrogen and phosphorus gave an average of 69 per cent more plant growth than the checks, whereas the virgin soils having a similar treatment gave 42 per cent more plant growth than the checks.

When 100 pounds of muriate of potash was applied per acre in addition to 400 pounds of 20 per cent superphosphate and 200 pounds of sodium nitrate, varied results were obtained. In many instances a decrease in yield was obtained when the complete fertilizer treatment was compared with the nitrogen-phosphorus treatment. The average for the complete fertilizer was 1 per cent increase on cropped soils and 6 per cent on virgin soils over the nitrogen-phosphorus series for the two crops. The virgin soils receiving a complete fertilizer gave 11 per cent more plant growth than the cropped soils with similar treatment for the crop of barley and 95 per cent for the crop of Sudan grass.

When the yields on the cropped and virgin soils are compared for each treatment it is noticeable that as the number of nutrients added increases the difference in yield between the two soils becomes less.

The difference in yield of the cropped and virgin soils was greater with the second crop than with the first. This supports the work of Millar (12) on the relative ability of cropped and virgin soils to renew the nutrient concentrations of the soil solution.

SUMMARY AND CONCLUSIONS

The chemical composition of cropped and the corresponding virgin soil of several different soil types was determined.

With the exception of nitrogen content, which, with one exception, was higher in the virgin soils, a comparison of analyses of cropped and virgin soils showed no consistent differences.

In many instances, wide differences in the quantities of certain elements are noted within a given soil type.

In the lighter soil types phosphorus has been decreased by cropping. In the heavier soil types phosphorus has been increased by cropping.

Exchangeable hydrogen, aluminum, manganese, calcium, magnesium, sodium, potassium, ammonium, absorption capacity, and pH were determined.

Very small differences were found in the exchangeable hydrogen of cropped and virgin soils.

There has been no change in pH due to cropping.

The content of the exchangeable bases calcium, magnesium, sodium, and potassium varies widely in the different soils.

Five types showed an increase in base exchange capacity and total bases in the cropped soils, and two showed a decrease.

The percentage of the total bases that are exchangeable increases as the soils become heavier.

As the texture of the soil becomes finer the relative proportion of the total bases and exchangeable cations constituted by calcium decreases.

With one exception the percentage of the total phosphorus readily available was greater in the virgin soils.

In all types studied the percentage of exchangeable calcium readily available was found to be greater in the cultivated soil. This indicates that the calcium is held more firmly by the absorbing complex of the virgin soils.

Pot experiments were conducted with these soils in the greenhouse.

The virgin soils gave greater plant growth under all treatments than did the cropped soils with similar treatments.

Virgin checks gave an average increase of 99 per cent plant growth over cropped checks for the two crops. Plant responses to phosphorus were in good agreement with laboratory determinations of readily available phosphorus.

The cropped soils gave the greater response to nitrogen fertilization.

Neither the cropped nor virgin soils responded to potassium fertilization. The difference in yield of the cropped and virgin soils was greater with the second crop than with the first.

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PLATE 1

PLANT GROWTH ON CULTIVATED AND VIRGIN SOILS

FIG. 1. Sudan Grass. 1 and 2 Plainfield, 3 and 4 Coloma, 5 and 6 Hillsdale, 7 and 8 College Farm; for each pair, cropped soil plus N-P-K on left; virgin soil plus N-P-K on right.

FIG. 2. Sudan Grass. 1 and 2 Plainfield, 3 and 4 Coloma, 5 and 6 Hillsdale, 7 and 8 College Farm; for each pair, virgin checks on left, cropped checks on right.

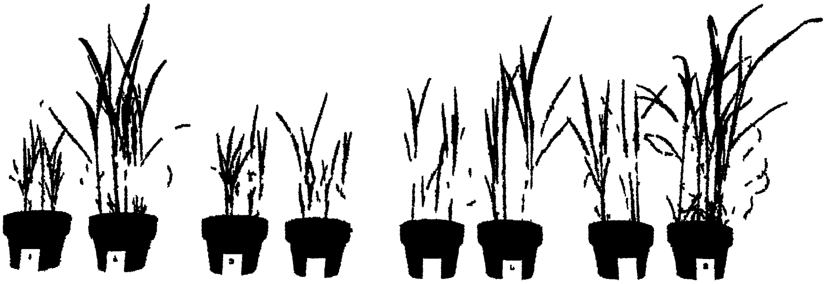

FIG. 1

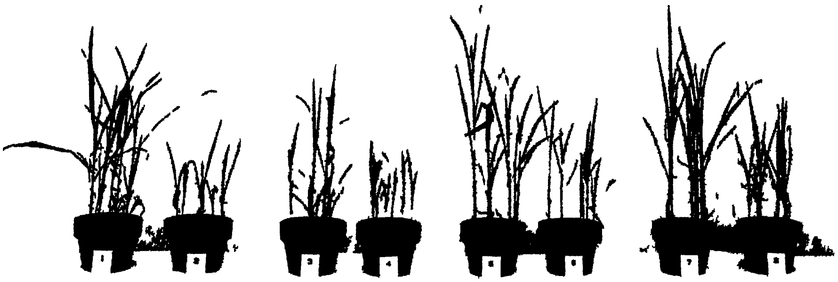

FIG 2

THE INFLUENCE OF A MULCH ON SOIL NITRATES

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In a previous paper[1] evidence of pronounced accumulation of nitrates under a mulch of waste hay and straw was presented. It was pointed out that we had no data which marked the course of nitrification during the period immediately following the application of the mulch, since the determination of nitrates was begun 3 years after the mulch was first applied.

Since it was a matter of practical importance as well as of scientific interest to determine the course of nitrification from the time of application of the mulch, another experiment was started in 1928 with this objective in mind. On May 3, 1928 a mulch of old hay was applied to the soil beneath the overhanging branches of three young Delicious apple trees in Block B of the station orchard. Additional mulch has been applied on top of the old mulch each spring from 1928 to 1931 inclusive. The rate of application has been from about 50 to 75 tons per acre of cured hay. As in the experiment on Block G-H previously reported, none of the mulch has been plowed under or worked into the soil by artificial means. Nitrates have been determined in composite soil samples. Methods of sampling and determination of nitrates described in the previous report have been followed in the present investigation. The original mulch experiment on Block G-H was continued, and the course of nitrate accumulation in the soil of this experiment was followed simultaneously with that in the new one. Results from both experiments are reported in table 1. The "unmulched" area of Block B was in sod, whereas the unmulched area of Block G-H was cultivated. The "mulched" areas of the two experiments had identical treatments.

DISCUSSION OF RESULTS

The data presented show that the course of nitrate accumulation in experiment G-H has continued similarly to that reported for the period 1925–26, that is nitrates have accumulated in much larger amounts under the mulched than in the cultivated soils. An exception is noted for the month of July, 1929. Also, in June and August of the same year nitrates were almost at the same level in the cultivated as in the mulched soil. A possible explanation of this deviation from the normal course lies in the abnormally low precipitation of that period. The precipitation for June, July, and August of 1929 at Amherst was 3.06, 0.70, and 1.54 inches respectively compared with normals of 3.54, 4.33, and 4.24 inches for the same period.

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By examining the data for Block B, the new experiment, it will be noted that no nitrates accumulated in 1928, the first year of the experiment, and that only traces and small amounts began to appear in 1929 and 1930, except that in July of 1930 a comparatively large amount of nitrate appeared in the mulched soil. In the winter of 1930–31 one of the trees of the experiment B was removed, but mulch was applied to the area where the tree had stood and the experiment continued as usual except that nitrates were determined for individual trees and for the area formerly occupied by the tree that was removed. It will be noted that nitrates appeared fairly regularly and often in large amounts in the mulched soil. Further, during the early period of growth

TABLE 1

Nitrates in mulched and unmulched soils

Expressed as parts per million NO_3 in dry soil

1928	7/21	8/10	8/28	9/11	9/24			
G-H mulched	107.9	49.1	3.6	..	..			
G-H cultivated	9.3	5.1	0	..	..			
B mulched	0	0	0	0	0			
B unmulched	0	6.2	0	0	0			
1929	**6/1**	**6/14**	**6/27**	**7/12**	**7/26**	**8/9**	**8/23**	**9/20**
G-H mulched	13.7	11.4	11.0	Tr.	18.9	50.4	50.7	81.2
G-H cultivated	6.8	5.6	10.8	8.3	19.7	42.1	4.9	4.5
B mulched	6.1	Tr.	0	Tr.	Tr.	Tr.	Tr.	5.6
B unmulched	Tr.	Tr.	0	Tr.	Tr.	Tr.	Tr.	Tr.
1930	**5/20**	**6/13**	**6/27**	**7/11**	**7/25**	**8/8**	**8/26**	
G-H mulched	42.3	5.4	31.1	56.7	57.1	70.2	39.1	
G-H cultivated	2.1	0	0	Tr.	Tr.	Tr.	0	
B mulched	0	0	0	0	87.7	0	0	
B unmulched	0	0	0	0	Tr.	Tr.	0	
1931	**6/16**	**6/30**	**7/14**	**7/28**	**8/11**	**8/18**	**8/25**	
G-H mulched	63.8	124.6	52.2	41.2	140.0	154.5	182.0	
G-H cultivated	5.8	35.2	14.0	17.7	15.5	Tr.	0	
B mulched*	2.8	0	24.1	0	27.4	31.6	Tr.	
B mulched†	26.1	68.0	59.8	74.8	166.1	104.0	79.3	
B mulched‡	12.0	Tr.	156.0	146.3	167.0	236.5	225.5	
B unmulched	0	0	Tr.	0	0	0	0	

* Individual tree on west.

† Tree removed preceding winter, but mulch applied.

‡ Individual east tree.

nitrates accumulated more rapidly in the mulched area from which the tree had been removed than in the areas still occupied by trees. We have no satisfactory explanation of the much higher amount of nitrates in the soil of the individual east tree than in that of the individual west tree; however it was determined that the lower, humified portion of the mulch of both areas ran very high in nitrates, the latter having 4,630 p.p.m. NO_3 and the former, 2,370 p.p.m. NO_3 on dry basis. Since it is entirely plausible that soil nitrates beneath the mulch are a result of leaching, and since leaching may vary from tree to tree or from one part of the mulch to another, the explanation may lie here. It is evident, also, that a slight variation in sampling with respect to amount of humified mulch incorporated in the soil sample may account for certain variations in nitrate content. In this latter way, the high figure for the mulched B soil of July 25, 1930 may be explained.

From the data submitted it is evident that under the conditions of our experiment 3 years were required for nitrates to accumulate in quantities sufficient to appear with noticeable regularity under the mulched soil. This does not mean that nitrification was not proceeding in the interim, but since young trees were growing vigorously on the area nitrates were no doubt used as rapidly as produced.

We have some unpublished data which indicate that in the time which elapsed between the applications of the mulch and the accumulation of an excess of nitrates in the soil, there has been a considerable narrowing of the carbon-nitrogen ratio in the mulch due to the processes of decay. It now appears that this action must occur for certain organic materials applied as a surface mulch in a way similar to that which occurs when organic materials are worked into the soil, before nitrification takes place rapidly enough to create a surplus. Some experiments are under way for the purpose of studying this question.

SUMMARY

Further evidence has been accumulated concerning conditions surrounding the accumulation of soil nitrates under a mulch of waste hay and straw applied to the surface of the soil under apple trees. None of the mulch was incorporated in the soil by plowing or working in any manner. During the first 3 years of the experiment nitrates accumulated to a slight extent only. During the fourth year after the mulch was applied nitrates accumulated rather consistently, and often in large amounts. The hypothesis is advanced that nitrification occurs mainly in the lower, humified layer of the mulch in contact with the soil rather than in the soil proper, and, by leaching, the nitrates are carried into the soil. It appears that nitrates accumulate only after the carbon-nitrogen ratio has been narrowed by the processes of decay acting on the organic matter of the mulch.

ON THE DISTRIBUTION OF ORGANIC MATTER IN THE SEA BOTTOM AND THE CHEMICAL NATURE AND ORIGIN OF MARINE HUMUS[1]

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Whatever information there is available concerning the abundance of organic matter in marine sediments or sea bottom is limited largely to the determination of loss on ignition. Only in a limited number of cases has the organic matter content been calculated from the total organic carbon. Because of its similarity in chemical composition and probably in the mechanism of its formation to soil humus or the total organic matter of land soils, the organic matter of the sea bottom may be designated as *marine humus.*

The marine humus is not uniformly distributed in the sea bottom, but varies considerably, depending on the distance from shore, bottom configuration, nature of sea bottom, etc. In general, the abundance of humus in the sea bottom has been reported to make up from 1 to 12 per cent of the total bottom material, on a dry basis, in the case of muds; much lower figures are usually reported for the humus content of sand bottoms. The abundance of humus in true oceanic deposits has still been insufficiently studied. If the loss on ignition is found to be inadequate as a measure of the humus content of the clay-rich material of the marine muds, which contain a considerable amount of chemically combined water and frequently also carbonate, it is especially true of the various oozes and other diatom, algal, and animal residues in pelagic formations. In order to avoid the errors thus involved, it was decided, therefore, to base the calculation of the humus content of marine sediments on the total organic carbon, as determined by dry or wet combustion methods.

In order to calculate the humus content from the amount of organic carbon found in the marine residues, it is essential to agree upon a certain factor. Because of a lack of sufficient knowledge concerning the chemical nature of this humus, however, this factor may still be considered as a matter open to dispute. In the case of normal field soils, the factor 1.724 is commonly used; it is based upon the assumption that the carbon content of soil humus is 58 per cent. Boysen-Jensen (2) suggested that the factor 2.0 is adequate for calculating the organic matter of marine muds, assuming that the marine humus contains 50 per cent of carbon. Since the carbon content of marine plant and animal residues, on an ash-free basis, is also about 50 per cent, one would have to assume, in order to justify the use of this factor, that there is no decided change in the balance of the various chemical constituents during the process of transformation of the organic matter from the residues of the plankton, algal material, and other marine forms of life into the marine humus.

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[1] Contribution No. 5, Woods Hole Oceanographic Institution, and Journal Series paper New Jersey Agricultural Experiment Station, department of soil microbiology.

[2] It is a pleasure to acknowledge here the indebtedness of the author to Dr. H. B. Bigelow and his associates at the Oceanographic Institution for the interest in this investigation and for considerable help variously rendered. The author is also indebted to Dr. H. W. Reuszer, Mr. C. E. Renn, and Mr. H. C. Stetson, for assistance in taking the samples and in certain analytical procedures, and to Dr. C. L. Carey, Mr. M. C. Allen, and Mrs. C. W. Hye for assistance in some of the chemical analyses.

It has been amply demonstrated (18), in the study of the decomposition of plant residues on land, that some of the organic constituents, like the sugars, cellulose, and certain hemicelluloses (40–44 per cent carbon), decompose rapidly; whereas others, like the lignins (62–64 per cent), decompose slowly; and still others, like the proteins (48–52 per cent carbon), may actually increase in total concentration, as a result of the synthesis of new proteins by microorganisms. As a result of this specific type of decomposition, there is a marked change in the carbon content of the residual humus as compared with that of the original plant residues. Probably similar transformations take place in the decomposition of plant and animal residues in the sea, no doubt of a different order of magnitude, due to a difference in the chemical nature of the residues, in the conditions under which decomposition is taking place and in the microörganisms which are active in the decomposition processes.

Certain relationships between the two most important elements of the marine humus, namely, the carbon and nitrogen, point to a similarity in chemical nature to land humus. Boysen-Jensen (2) found that apparently pure sand of the North Sea contained, on a dry basis, 0.34 per cent carbon and 0.027 per cent nitrogen, with a carbon-nitrogen ratio of 12.6. The carbon content of the marine bottom material from the various parts of the Limfjord varied from 0.58 to 4.3 per cent, with a C:N ratio of 10.6 to 12.4. The clay bottom of the Kattegat, at depths of 30 to 35 m., contained 2.3 per cent carbon, with a C:N of 11.5, while the mud of the inner part of the Roskilde Fjord contained as much as 10.2 per cent carbon. At greater ocean depths, namely, at 800 to 2,200 meters, Boysen-Jensen, using eight samples obtained from the Ingolf expedition, found 0.3 to 1.6 per cent carbon, with a C:N of 10.2. One may conclude from these results and those of Petersen and Boysen-Jensen (7) that the organic matter content of the sea bottom is very variable; however, the carbon-nitrogen ratio of the organic matter is more or less constant, varying from 8.1 to 12.6, with an average of 11.1. If one assumes that the carbon content of the marine humus is 50 per cent, the nitrogen content will thus be found to be about 5 per cent.

Moore (4) found that the organic matter content of marine mud, as measured by the total nitrogen, changes with depth of the deposit. In the case of the muds and sandy muds of the Clyde Sea area, the nitrogen content varied from 0.089 to 0.379 per cent at a depth of 0 to 5 cm., from 0.083 to 0.330 per cent at 5–10 cm., from 0.074 to 0.295 per cent at 10–15 cm., and from 0.074 to 0.302 per cent at 15–20 cm. depth. The decrease with depth is gradual, but not very appreciable. In a few instances there was an increase in the nitrogen content with the depth of mud. No correlation was observed, however, between the depth of the water and the nitrogen content of the mud.

In a series of determinations of the organic carbon content of marine sediments, Trask (15, 16) reported a range from 0.2 per cent, for a sand bottom, to 4.2 per cent, for a clay bottom, with an average of 1.8 for 25 near-shore sediments and 2.1 per cent for Channel Island deposits (California). The organic carbon content of sediments of past geologic ages was found to be similar to that of recent sediments. The C:N ratio of a number of representative sediments from all over the world was 8.4 ±1.0, with a range of 5.3 to 13.3. Recent marine deposits contain more nitrogen in respect to carbon than do land soils and lacustrine deposits. The C:N ratio of deposits of past geologic ages was found to be greater than that of recent deposits. Trask concluded that the organic matter content of the sea bottom is influenced by the bottom configuration; because of its greater ease of transportation, it increases in concentration as the texture becomes finer. Deposits in depressions and in closed basins contain more organic matter than do those on adjoining ridges and slopes. Trask also found a rapid drop in the organic matter content of the marine sediments with depth, the drop becoming less with an increase in depth; he emphasized the fact, however, that since this depends on the rate of deposition, the organic matter content may be sometimes greater at lower depths than at the surface.

The very surface layer of the mud or of other marine bottom material is rich in detritus, namely, plant and animal residues in the process of decomposition, and in the so-called jelly-like material (8). This detritus contains an abundant bacterial population (21) and is different in nature from the true marine humus or the organic matter which has already undergone extensive decomposition and has become an integral part of the bottom material. Murray and Hjort (6) suggested that the organic substance of the marine deposits serves as a fertilizer for the surface layers of the marine bottom. Petersen (7) also believed that the mud-eating animals obtain nutrients from the surface layers of the mud. It still remains to be determined, however, to what extent true marine humus can offer nutrient material to animal life.

The few available determinations on the abundance and chemical nature of organic matter in the bottom of deep seas are based almost entirely on the loss-on-ignition method. In the case of the Globigerina ooze, figures ranging from 0.8–6.0 per cent have been found, the Challenger expedition reporting 3.6 to 10.4 per cent; red clay was found to give 2.7 per cent, by the loss on ignition method, Radiolaria ooze — 4.3 to 7.4 per cent, and mud of Diatomaceae — 4.0 per cent (1).

As to the chemical nature of the marine humus, the few investigations of Boysen-Jensen, in which he tried to establish the fact that Zostera contributes largely to the formation of this humus, and of Trask and Hammar, who found a similarity in chemical composition between the soil humus and marine humus, practically exhaust all the available information.

The following experiments represent an attempt to study the abundance of humus in the marine bottoms, its nature and possible origin, and its further decomposition.

EXPERIMENTAL

Abundance of humus in the marine bottom, especially in marine muds

The humus content of the marine bottom was calculated from the amount of organic carbon found. This carbon was determined by the use of Tiurin's (14) modification of Schollenberger's method. The actual amount of dry material taken for analysis does not play any important part in the determination, as shown by the results reported in table 1, on four different types of marine mud with varying humus content and one sample of humus isolated from the mud by treatment with dilute alkali solution and precipitation with acid. Several of the mud samples, as well as certain humus preparations isolated from land soils, were analyzed for total carbon both by the regular combustion method, using an electrically heated furnace and absorbing the CO_2 liberated, and by the simplified oxidation procedure. The results obtained by the two methods checked up very well.

The nitrogen determinations were made by the usual Kjeldahl method using the air-dry bottom material; it was found later (20), however, that in some instances some of the nitrogen may be lost in the process of drying of the material, especially in the case of surface layers of mud or recent formations. In some cases, the loss on ignition was also determined using the common procedure of igniting the oven-dry material to constant weight.

The following investigations were based largely upon the study of the humus content of mud collected from Buzzard's Bay, from Cape Cod Bay, and from several stations in the Gulf of Maine. The samples of mud were obtained by three different methods: (*a*) by the use of a small glass cover similar to the one described by Moore and Neill (5), (*b*) by means of a heavy brass pipe, used extensively at the Oceanographic Institution, (*c*) by means of a Petersen grab. The organic matter content of marine sand bottoms was studied only to a limited extent. A few samples of bottom material were made available from the deeper layers of the sea, through the Wilkins expedition and a cruise of the "Atlantis" to Bermuda.

The mud samples from the aforementioned stations belong to the group usually described as "blue muds," formed by terrestrial or semi-pelagic sedimentation. The color of the mud is due to the organic matter and the FeS content. Frequently the large profiles of mud from the Gulf of Maine, a meter or so in depth, showed a marked change in color with a change in depth. The samples of mud were allowed to dry, usually at temperatures not above 85–90°C. The results of analysis are based upon the total dry mud, not freed from the sea salt.

TABLE 1

Carbon content of marine mud, as determined by the simplified oxidation procedure

NATURE OF MUD SAMPLE NUMBER	AMOUNT OF DRY SAMPLE USED*	TITRATION RESULTS	EQUIVALENT TITRE	CARBON CONTENT
	mgm.	*cc.*	*cc.*	*per cent*
0	0	30.2	0	0
1	300	20.3	9.9	1.98
1	500	13.9	16.3	1.96
2	300	21.7	8.5	1.70
2	500	15.5	14.7	1.76
3	465	18.4	11.8	1.52
3	382	20.5	9.7	1.52
4	539	25.9	4.3	0.48
4	661	25.3	4.9	0.45
α-humus	20	13.4	16.8	50.4
α-humus	50	4.5	40.8	49.2

* Ten cc. of the chromic acid solution was used for all the mud samples and 15 cc. for the two humus samples.

It was essential to determine first how uniformly the humus is distributed in marine sediments, not only vertically but also horizontally. A series of samples of mud were taken from three stations in the Gulf of Maine; the water at those stations was 190 to 225 m. deep; the location of these stations and the results of the bacteriological investigation of the water, as well as of the mud itself, are reported in detail elsewhere (20). Several samples of mud were taken from each station, by means of the small glass corer, as close to one another as the movement of the boat made possible; these samples represent only the surface 10 to 20 cm. of mud. The results reported in table 2 show that, in general, there is very little variation in the horizontal distribution of the humus in the mud, when the samples are not taken too far apart as shown by the carbon and nitrogen content, as well as by the carbon-nitrogen ratio.

However, at greater distances, even only a few miles, the samples of mud may vary considerably in humus content, as shown in tables 3 and 4, where the humus content of the muds in the vicinity of Woods Hole are reported. The method of determination of humus by loss on ignition gives results which are considerably in excess over the total organic matter, as calculated from the total carbon and nitrogen; there is, however, a very definite parallelism between the two sets of data. Those samples of mud (sample 1 and surface portion of sample 5) which give the highest loss on ignition also contain the largest amount of carbon and nitrogen.

TABLE 2

Distribution of marine humus in sea mud, as shown by the carbon and nitrogen content

Per cent of dry material

STATION NUMBER	SAMPLE NUMBER	CARBON CONTENT	NITROGEN CONTENT	C/N
1329	1	2.46	0.284	8.7
1329	2	2.50	0.290	8.6
1329	3	2.48	0.290	8.5
1329	4	2.46	0.282	8.7
1329	5	2.37	0.278	8.6
1329	Average	2.45	0.285	8.6
1330	1	2.65	0.304	8.7
1330	2	2.66	0.284	9.4
1330	3	2.62	0.306	8.6
1330	4	2.70	0.294	9.2
1330	5	2.81	0.288	9.7
1330	Average	2.69	0.295	9.1
1331	1	1.58	0.156	10.1
1331	2	1.54	0.148	10.4
1331	3	1.55	0.144	10.8
1331	4	1.36	0.133	10.2
1331	5	1.29	0.133	9.7
1331	Average	1.46	0.143	10.2

When samples are taken from the same location, at different periods, varying results may be obtained (table 4). A series of samples of mud were collected from Cape Cod Bay, at three different intervals. Some of the samples were taken within the bay itself, between Race Point and end of the canal, and some near the Cape Cod Lightship, at definite distances from shore, station 5 being nearest to the shore (10 miles) and station 8 farthest from the shore (25 miles). Considerable variability was found in the humus content of the mud taken presumably from the same station, but at different periods. The reason for

TABLE 3

Humus content of marine muds in Buzzard's Bay

NUMBER	LOCATION, NEW BEDFORD HARBOR	MOISTURE IN FRESH MUD	ON PER CENT BASIS OF DRY MATERIAL			C/N
			Loss on ignition	Carbon	Nitrogen	
		per cent				
1	Above harbor	54.6	7.45	2.30	0.226	10.2
2	Center of harbor	49.4	5.90	1.96	0.188	10.4
3	Below harbor	39.0	5.25	1.76	0.164	10.7
4	Sandy part of harbor	23.0	1.85	0.58	0.047	12.4
5 Upper 4 cm.	Near sewer outlet	55.0	7.65	3.37	0.250	13.5
5 Lower 4 cm.	Near sewer outlet	24.3	3.50	1.21	0.090	13.4

TABLE 4

Humus content of marine mud in Cape Cod Bay and near Cape Cod Lightship

STATION NUMBER	DATE OF SAMPLING	CAPE COD BAY			
		Moisture content of fresh mud	Humus in dry material		
			Carbon	Nitrogen	C/N
		per cent	*per cent*	*per cent*	
1	VIII/24/32	29.6	0.82	0.078	10.5
2	VIII/24/32		2.98	0.306	9.7
3	VIII/24/32	44.0	1.84	0.180	10.2
4	VIII/24/32	53.6	2.51	0.206	12.2
6	VIII/24/32	67.4	3.04	0.266	11.4
4	IX/20/32	52.5	2.36	0.226	10.5
6	IX/20/32	64.4	3.92	0.325	12.1
		CAPE COD LIGHTSHIP			
6	VII/13/32	58 8	1.42	0.147	9.7
7	VII/13/32	53.3	1.90	0.206	9.2
5	VIII/24/32	25.0	0.34	0.042	8.1
7	VIII/24/32	54.8	1.55	0.160	9.7
8	VIII/24/32	56.6	1.73	0.206	8.4
6	IX/20/32	53.9	1.99	0.166	12.0
7	IX/20/32	63.4	2.22	0.236	9.4
8	IX/20/32	66.3	2.32	0.257	9.0

this variability is to be looked for primarily in the following two causes: (*a*) the non-uniformity of the sea bottom and (*b*) the fact that the organic matter content of the surface of the mud varies from that of the lower layers and that the surface layer is apt to be changeable, because of water movements and depositions of fresh material. This is especially true of such near-shore stations as these represent. The carbon-nitrogen ratio of the humus in the muds from Buzzard's Bay was found to range from 10.2 to 13.5 and in the humus of muds from Cape Cod Bay, from 8.1 to 12.2. The latter variation is almost exactly the same as that reported by Petersen.

Schokalsky (9) measured the stratification of the muds at the bottom of the Black Sea. He suggested that the alternating layers found there are probably a result of a winter terrigenous deposit (gray) and a spring and summer planktonic deposit (black). This phenomenon enabled him to calculate that it takes 50 years to deposit a layer of mud 1 cm. in thickness. Similar stratification was reported by Moore (5) for the mud in the Clyde Sea area.

TABLE 5

Abundance of marine humus in muds deposited at different ages

STATION NUMBER	DEPTH OF MUD IN PROFILE	MOISTURE CONTENT OF FRESH MUD	ON PER CENT BASIS OF DRY MATERIAL		C/N
			Carbon	Nitrogen	
	cm.	*per cent*			
1329	0–30	65.0	2.52	0.290	8.7
1329	30–60	60.8	2.45	0.250	9.8
1329	60–90	58.8	2.32	0.238	9.8
1330	0–30	59.9	2.74	0.296	9.3
1330	30–60	58.2	2.75	0.254	10.8
1330	60–90	57.4	2.60	0.236	11.0
1331	0–35	50.0	1.59	0.148	10.7
1331	0–35	49.5	1.56	0.132	11.8
1331	35–65	46.9	1.64	0.130	12.6
1331	65–90	48.8	1.61	0.128	12.6
1331	90–115	46.9	1.57	0.126	12.4
1331	90–115 (reddish mud)	46.8	1.04	0.080	13.0
1332	0–25	31.2	0.67	0.050	13.4
1332	25–50	36.7	1.12	0.092	12.2
1332	50–75	37.7	1.43	0.096	14.9

In order to determine whether there is a marked difference in the humus content of the mud deposited at different ages, several profiles were obtained, by the use of the large brass borer, from the Gulf of Maine (20). The length of these cores when brought up to the surface ranged from 75 to 112 cm.; they were probably deeper in the natural state, since they have undergone some compression by the sampler. The cores were divided into three or four sections and, as soon as brought to the laboratory, dried and analyzed. The humus content of the mud taken from the different sections varies considerably from station to station, as shown in table 5. The variation with depth is more uniform. In the case of the first three profiles (stations 1329–1331), there is a gradual but slow reduction in the humus content with an increase in the depth of mud; however, in the fourth profile (station 1332), there was an increase in humus content with depth. In the case of the last profile, the inorganic particles of the surface layers of mud were decidedly coarser and became finer with an increase in depth. This was not the case of the other three profiles. The mechanical analyses of the mud profiles from station 1329 (fig. 1) and 1332 (fig. 2) were made by Mr. Stetson of the Oceanographic Institution (3), to whom the author wishes to express his sincere indebtedness.

The carbon-nitrogen ratio of the humus was found to increase gradually with an increase in the depth of mud; this is probably because under the anaerobic conditions prevailing in the mud an increase in depth results in a greater, even if only very slow, decomposition of the nitrogenous than of the non-nitrogenous constituents.

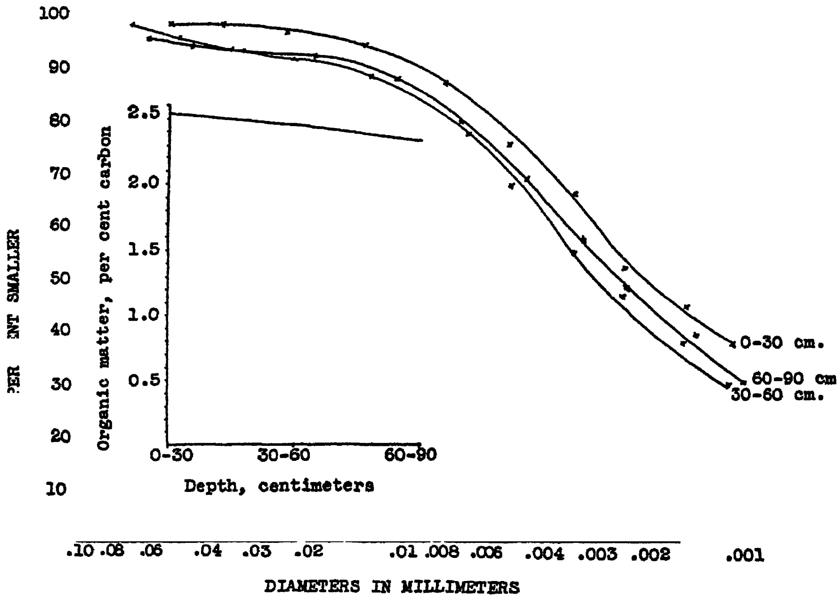

FIG. 1. CHANGE IN MECHANICAL COMPOSITION, AS DETERMINED BY HYDROMETER METHOD, AND HUMUS CONTENT, AS MEASURED BY ORGANIC CARBON, WITH DEPTH OF MARINE MUD.—STATION 1329

Only a few samples of bottom material obtained at greater distances from shore were available for determinations of the humus content. Table 6 shows the results of analyses of a mud profile brought by Sir Wilkins from the Nautilus expedition. This profile was taken north of Spitzbergen at 81–52 Lat. N. and 13.40 Long. E., at a depth of 1,620 m. The length of the core, in the compacted form, was 42 cm. The total organic matter content of this mud was lower than that of the mud in the Gulf of Maine; there was also a gradual decrease in humus content with an increase in depth of the mud, as shown both by the carbon and nitrogen data. It is of interest to note particularly that the carbon-nitrogen ratio of the humus in this mud as well was 10.65 to 12.44. Table 7 shows the abundance of humus at great depths of water, truly oceanic in nature. These samples of bottom material were taken during the "Atlantis" cruise to the Bermudas in September, 1932. The humus content of the sediment is rather low, ranging from 0.60 to 0.78 per cent, calculated on the basis of the total organic carbon (using the factor 2.0). The loss on ignition of these samples was very high; in the case of station 1359, a loss of 20.2 per cent

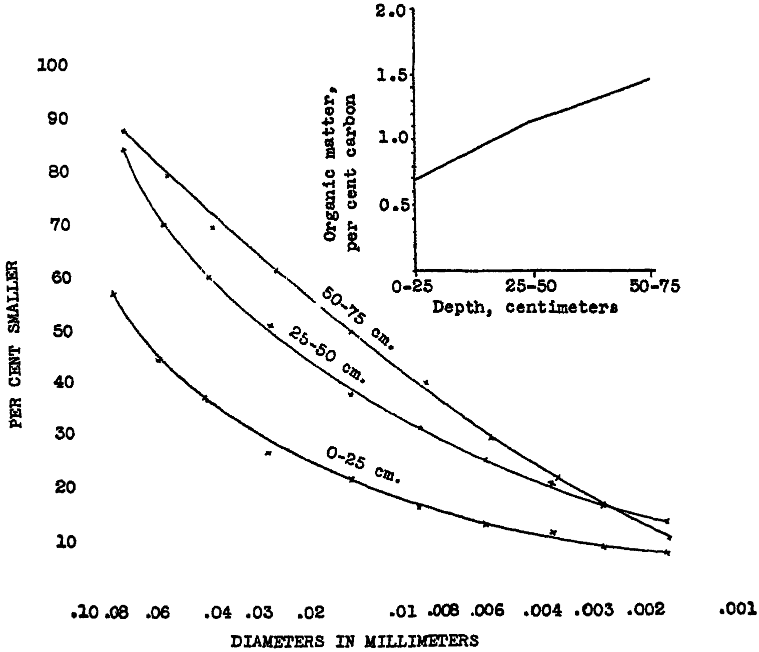

FIG. 2. CHANGE IN MECHANICAL COMPOSITION, AS DETERMINED BY HYDROMETER METHOD, AND HUMUS CONTENT, AS MEASURED BY ORGANIC CARBON, WITH DEPTH OF MARINE MUD.—STATION 1332

TABLE 6

Marine humus in mud from north of Spitzbergen

On basis of dry material

DEPTH OF MUD	ORGANIC CARBON	TOTAL NITROGEN	C/N
cm.	*per cent*	*per cent*	
0–14	0.852	0.080	10.65
15–28	0.672	0.054	12.44
29–42	0.468	0.044	11.10

was obtained on the basis of the dry material, as contrasted with 0.746 per cent as calculated from the organic carbon.

The humus content of sandy bottoms is considerably lower than that of muds and even of deep sea bottoms. Two samples of sand bottom were taken (20) at George's Bank (samples 1335 and 1336); the water at those stations was 74 and 64 meters deep, respectively. The organic carbon of the two samples of sand bottom was found to be 0.084 and 0.120 per cent and the nitrogen 0.008 and 0.009 per cent, respectively; the C:N ratio of the two samples was, therefore, 10.5 and 13.3.

The results of the quantitative analyses of the humus content in marine bottoms permit the drawing of the following conclusions: (*a*) The organic matter content of marine bottoms varies considerably, even at a distance of a few miles from one another and frequently in close proximity, as shown both by the organic carbon and total nitrogen content; (*b*) in most instances, there is a slow and gradual diminution of the amount of humus present in the mud with an increase in depth, although in some instances the reverse may hold true; (*c*) the carbon-nitrogen ratio of the humus in the mud is more or less constant, namely, about 10:1; the tendency is for this ratio to be narrower in muds containing a high content of humus and wider in muds with a low humus content; this ratio was found to range between 7.5:1 and 14.9:1; (*d*) the humus content of sand bottom and deep sea deposits is considerably less than that of mud bottoms; (*e*) a definite relation seems to exist, to a certain extent at least, between the size of the inorganic particles in the sediment and the amount of humus, the latter increasing with a decrease in size of the former.

TABLE 7

Humus content of the true oceanic bottom

STATION NUMBER	SAMPLE NUMBER	DEPTH OF BOTTOM	CARBON CONTENT	NITROGEN CONTENT	C/N
		m.	*per cent*	*per cent*	
1348, lat. 39°36′ N long. 64°52′ W	B T 2	4,800	0.348	0.042	8.3
1351, lat. 39°37′ N long. 63°50′ W	B.T 3	5,040	0.432	0.043	10.0
1359, lat 32°12′ N long 64°36′ W	B T. 5	2,720	0.373	0.032	11.7
1363, lat. 33°39′ N long. 67°28′ W	B.T. 6	5,400	0.456	0.061	7.5

Chemical nature of marine humus

Our knowledge of the chemical nature of marine humus is very limited. Still less is known concerning the mechanism of its formation, and there is no information whatsoever which would enable us to state whether the organic matter in marine muds is of the same chemical nature as that of sand bottoms and deep oceanic deposits or not. The fact that the organic matter of marine sediments forms only a small portion of a system predominantly inorganic in nature, the fact that it is formed from plant and animal residues, and through the agency of microörganisms, and further its specific physical (color) and chemical properties (carbon content, nitrogen content, and C:N ratio) would justify a comparison of the organic matter in marine bottoms, or marine humus, with that of the organic matter in land soils or soil humus. However, differences between the chemical composition of the residues of land plants and marine plants, as well as differences in the nature of the microörganisms active in the decomposition processes in land soils (fungi, actinomyces, bacteria, and animals) and in sea water and marine bottoms (bacteria and animals only) would lead one to expect certain differences in the chemical nature of this humus. In the case of soil formations, the humus of mineral soils varies considerably from that of forest soils and especially from that of peat bogs; one may also expect that the humus of mud bottoms should vary in chemical nature from that of sand bottoms and deep ocean sediments, as a result of difference in origin and decomposition processes.

In the study of the chemical nature of humus in land soils, chemists have assumed that this humus consists of a few specific "humic acids"; some went as far as to claim that humus is comparatively simple in chemical composition. However, as a result of the investigations of Schreiner and Shorey (11) at the Bureau of Soils in Washington, it was shown that numerous compounds can be isolated from the various so-called "humic acid" fractions of the soil humus. The writer and his associates have also shown (18, 19) that although the humus of land soils contains small amounts of fatty and waxy substances, of carbohydrates, especially hemicelluloses, and of other organic compounds, it consists chiefly of two complexes, namely, proteins and lignins. The last two groups make up nearly 75 per cent of the total humus of the soil.

On treatment of soil with dilute alkali solutions, a large part of the total humus, frequently 70 to 75 per cent, can be dissolved. When this solution is acidified with mineral acid, a heavy precipitate is obtained which is insoluble in acid. This fraction represents only a part of the humus brought into solution by the treatment with dilute alkalies. When the acid filtrate is adjusted to neutrality by means of an alkali, another precipitate is obtained, which is largely inorganic in nature. The first precipitate was designated by the writer (17) as "α-humus;" it is commonly known in the literature as "humic acid" or "humic fraction." The second fraction was designated as "β-humus"; this is frequently considered as a part of the "non-humic fraction" of the humus. The α-humus fraction of the soil humus contains only about 1 to 3 per cent ash, 55–58 per cent carbon and 2.5–3.5 per cent nitrogen; the β-humus fraction consists of 65–80 per cent of inorganic material (largely aluminum silicate) and of a smaller part of organic matter.

A procedure similar to the one outlined in the foregoing was used for the study of the humus in marine muds, in an attempt to elucidate the chemical nature of this humus and compare it with that of humus in field soils. A quantity of mud was collected in Buzzard's Bay, near Wepecket Island, using the Petersen grab. The dry mud analyzed as follows:

	per cent
Loss on ignition	7.18
Total organic carbon	2.01
Carbonate carbon	0.15
Total nitrogen	0.23
Carbon-nitrogen ratio	8.72

Four-hundred-gram portions of the air-dry mud were extracted with 400-cc. portions of a 4 per cent NaOH solution, for 1 hour, at 15 pounds pressure. The extracts were filtered through paper and the residues again extracted with additional quantities of 200 cc. of the alkali solution for 30 minutes at 15 pounds pressure, and again filtered. The residues after the second extraction were washed twice with distilled water. The combined filtrates and washings were neutralized in the cold with a 1:1 hydrochloric acid solution; an excess of the acid, equivalent to about one-half of the amount used for neutralization, was then added. A heavy, dark-colored precipitate was produced; it was removed by filtration through paper, washed with distilled water until free from acid, and dried. This precipitate may be designated as the "α-fraction," to make it comparable to the similar fraction obtained by the same procedure from land soils. The acid filtrate from the α-fraction was now neutralized to litmus with 40 per cent NaOH solution, to a pH of about 4.8. A heavy precipitate was again formed, which was filtered off, washed, and dried; this fraction formed a light colored powder and was designated as β_1. The filtrate from the β_1 fraction was now treated with concentrated $CaCl_2$ solution, which resulted in another heavy precipitate; this fraction was also removed by filtration, washed, dried, and designated as the "β_2;" it was also very light in color. The filtrate from the β_2 fraction was treated with two volumes of 95 per cent ethyl alcohol. A heavy precipitate was formed, which was allowed to settle, and was removed by centrifuging. It was washed with 95 per cent alcohol and acetone and dried over sulfuric acid. A light-brown colored preparation was thus obtained, which was designated as the "γ-fraction" of the marine humus.

The composition of the four fractions prepared from 400 gm. of air-dry marine mud is given in table 8, and the total nitrogen and carbon balances are given in tables 9 and 10. The extraction of the mud with 4 per cent alkali solution, at 120°C., removed 84 per cent of the total organic matter, or humus in the mud, as measured by the nitrogenous constituents, and only 70 per cent of the humus, on the basis of the relative amount of organic carbon extracted. Nearly a half of this humus, as measured by the carbon content, was recovered in the α-fraction, or the acid precipitate of the alkali extract. A large part of the nitrogen of the organic matter in the mud is left, however, in the filtrate.

TABLE 8

*Yield and chemical composition of the four fractions obtained from marine mud by alkali extraction**

Per cent of dry material

		β_1	β_2	
Total yield, *gm*	5.081	8.997	10.789	3.280
Ash in dry material, *per cent*	4.9	89.4†	75.5†	52.1
Organic carbon in dry material, *per cent*	54.5	3.36	4.99	22.1
Nitrogen in dry material, *per cent*	4.32	0.36	0.53	2.46

* From 400 gm. of dry mud.

† The high carbonate content of these two fractions, especially of β_2, accounts for the discrepancy between the loss on ignition and the organic carbon content.

TABLE 9

Nitrogen balance in 400 gm. of dry marine mud

	mgm.
Total nitrogen in mud used for extraction	920
Nitrogen in residual mud, not extracted by the alkali solution	148
Nitrogen extracted by 4 per cent NaOH solution	772
Nitrogen accounted for, α-fraction	219.5
β_1-fraction	32.4
β_2-fraction	57.2
γ-fraction	80.7
Nitrogen in final filtrate	271.3
Total nitrogen accounted for	661.1
Nitrogen not accounted for (largely lost as NH_3 in the process of extraction with hot alkali solution)	110.9

TABLE 10

Organic carbon balance in 400 gm. of dry marine mud

	mgm.
Total organic carbon in mud	8,010
Organic carbon in residual mud, after NaOH extraction	2,412
Carbon extracted by 4 per cent NaOH solution	5,598
Carbon accounted for in α-fraction	2,769
β_1-fraction	302
β_2-fraction	539
γ-fraction	725
Total carbon accounted for	4,335
Carbon not accounted for in 4 fractions and present largely in the filtrate from the γ-fraction	1,263

By subtracting the ash from the total dry materials of the α-fraction and assuming that all the nitrogen is in the form of protein, with 16 per cent nitrogen and 50 per cent carbon, one can divide the organic matter of this fraction into two complexes; namely, the protein complex, which makes up 27 per cent of the total α-fraction, and another complex making up the remaining 68.1 per cent of the fraction which is non-nitrogenous and which contains 60.2 per cent carbon; this high carbon content is characteristic of lignin complexes, as shown later. One can conclude, therefore, that the α-fraction is largely a ligno-protein complex. The β_1 and β_2 fractions contain only a small amount of organic matter and can be left out of further consideration.

The organic matter of the γ-fraction contains 46.2 per cent carbon and 5.14 per cent nitrogen. By assuming that the nitrogen is in the form of protein or its derivatives and by allowing 16 per cent nitrogen and 50 per cent carbon for the protein, one finds that the non-nitrogenous part of the organic matter of the γ-fraction contains 43.6 per cent carbon. This would place this fraction among the carbohydrates. On treating some of the dry γ-fraction with a hot dilute mineral acid, a considerable amount of reducing substance was formed. By boiling it with 12 per cent hydrochloric acid and collecting the CO_2 in standard $Ba(OH)_2$ solution, then titrating back with standard oxalic acid solution, a large amount of uronic acid anhydride could be demonstrated. The uronic acid content of the original mud was 0.219 per cent, and that of the γ-fraction was 4.1 per cent. A large part of the uronic acid present originally in the mud was thus found in the γ-fraction. Some of the uronic acid was destroyed on treatment of the mud with the hot alkali solution. The presence of uronic acid complexes in marine sediments can easily be understood, since polyuronides form important constituents of many of the marine algae (21).

A detailed analysis of the proximate chemical composition of the α and γ fractions, isolated from marine mud by the procedures outlined, is given in tables 11 and 12. These results show that the α-fraction is largely a ligno-protein complex, the total lignin and protein accounting for 85.90 per cent of the total organic matter in this fraction. On treatment of the α-fraction with 80 per cent sulfuric acid for 2 hours in the cold, then diluting with 15 volumes of water and boiling for 5 hours, 78.8 per cent of the total α-fraction was left unhydrolized. The remaining 14.1 per cent of the organic substance in the α-fraction is made up of certain carbohydrates, which give on hydrolysis with acids reducing sugars and non-reducing compounds; this is further confirmed by their carbon content. The α-fraction obtained from marine mud is thus found to be very similar in chemical composition to the same fraction obtained from field and garden soils, with the exception that it is considerably higher in protein; the corresponding fraction from soil usually contains 3.0 to 3.5 per cent nitrogen, against 4.54 per cent in the fraction from marine mud. The higher relative nitrogen content of the mud and of its α-fraction, as compared with field soils, is probably due to the fact that a much larger part of the organic matter in marine mud is formed from animal residues, which are higher in protein, than is the case of field soils.

The chemical nature of the γ-fraction is distinctly different from that of the α-fraction. It represents largely a protein-carbohydrate complex, with a high uronic acid content. There is no doubt that the uronic acid compounds of this fraction were derived from the plant residues (algae, diatoms) in the ocean, which have resisted decomposition by bacteria, or from the slimy material synthesized by bacteria and algae. The fact that the humus of field

TABLE 11

Proximate chemical composition of α-fraction of marine humus

	DRY, ASH-FREE MATERIAL
	per cent
Total carbon	57.30
Total nitrogen	4.54
Protein equivalent of nitrogen	28.38
On treatment with cold 80 per cent sulfuric acid; sugar, as glucose	5.26
Ligno-protein fraction (unacted upon by 80 per cent sulfuric acid)	78.80
Protein content of "lignin fraction"	21.28
"Lignin" content of α-fraction	57.52
"Lignin" content of organic matter of α-fraction	60.55
Carbon as protein in α-fraction (on basis of 50 per cent carbon)	14.19
Carbon as hemicellulose yielding sugar	2.10
Carbon as lignin (64 per cent carbon)	36.81
Total carbon accounted for	53.10
Carbon not accounted for (57.30 − 53.10)	4.20
Total organic matter not accounted for [100 − (28.38 + 5.26 × 0.9 + 57.52)]	9.4
Carbon content of organic fraction not accounted for, and dissolved by boiling dilute acid (4.20 × 100:9.4)	44.7

TABLE 12

Proximate chemical composition of γ-fraction of marine humus

	DRY ASH-FREE MATERIAL
	per cent
Total carbon	46.20
Total nitrogen	5.14
Protein equivalent	32.1
Total uronic acid anhydride	8.50
On treatment with cold 80 per cent sulfuric acid, fraction was completely dissolved, except 3.5 per cent, which was practically all ash; reducing sugar formed, as glucose	21.80

soils and peats contains a high amount of uronic acid complexes has been pointed out elsewhere (19); the presence of uronic acid in soil humus has actually been demonstrated by Shorey (12). The large amount of reducing sugar obtained on hydrolysis of the γ-fraction points to the presence of other carbohydrates, besides the uronide in this fraction.

Among the various specific chemical complexes of marine humus, the pentosans received special consideration. Boysen-Jensen (2) reported that pentosan makes up 0.27 to 1.06 per cent of the total organic matter, the pentosan carbon comprising 5.2 to 8.7 per cent of the total carbon. Recent studies (12, 19) on the soil organic matter brought out the fact that the complex formerly known as pentosan and determined by the furfuraldehyde method, is largely a uronic acid compound. In light of these investigations, one may suggest that the "pentosan" of Boysen-Jensen is probably a carbohydrate derived to a large extent from the uronic acid complexes in the marine humus.

These analyses, even if based only upon the humus of one sample of marine mud, permit one to conclude that this humus has a specific chemical composition. The humus constituents can be classified into three groups: (*a*) lignins and their derivatives, (*b*) proteins, and (*c*) carbohydrates, largely polysaccharides and polyuronides. One can also demonstrate the presence of a small amount of fatty and waxy substances, as well as other chemical constituents in lower concentrations. There is no doubt that the humus in sand bottoms and especially in the deep ocean sediments may vary in chemical composition from that of muds, and possibly that the humus in other marine muds may vary from that of the mud used for this study, but this is just as one would expect it.

Factor for calculating the humus content of sea bottom sediments

It was pointed out previously that the use of the factor 2.0 for calculating the total organic matter or humus content in marine bottoms from the amount of organic carbon found is open to criticism, because insufficient attention is paid to the processes of humus formation and to its chemical nature. However, one would hardly be justified in adopting the factor 1.724 commonly used for the calculation of humus in land soils, without further investigation, because of the marked difference in chemical composition between marine plants and land plants; animal residues and excreta contribute of course to both formations. There is no doubt that marine humus in muds which are not very far distant from shore is partly of terrestrial origin, as a result of which one can expect to find in the humus a fraction of a ligno-protein nature (α-fraction) with a high carbon content.[3] However, since the residues of marine plants which consist largely of carbohydrates with a low carbon content also contribute to the marine humus, one would expect that these would tend to lower the carbon content of the humus. The isolation of the specific fractions described in the foregoing may enable one to make certain calculations which will tend to throw a definite amount of light upon this problem.

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[3] The decomposition of the Zostera plants growing close to shore will no doubt also contribute a similar complex.

The treatment of the mud with 4 per cent NaOH solution resulted in the removal of 70 per cent of the organic matter from the mud, as calculated from the carbon content. Of this organic matter, just one-half, or 35 per cent of the total carbon, was recovered in the α-fraction. The lignin made up 60 per cent of this fraction; in other words, 21 per cent of the total organic matter in the humus soluble in the hot NaOH solution, under pressure, is in the form of lignin-like complexes. The total protein content of that part of the humus which was soluble in the alkali solution, as calculated from the amount of nitrogen extracted, makes up 30.2 per cent of the total humus, assuming a carbon content of 50 per cent. The remaining 18.8 per cent of the dissolved organic matter is made up partly of carbohydrates (hemicelluloses) and related compounds and partly of various other complexes the exact chemical nature of which is not known. The carbon content of the humus extracted with 4 per cent NaOH solution can therefore be calculated as follows:

COMPLEX	PER CENT OF TOTAL HUMUS	THEORETICAL CARBON CONTENT	CARBON FRACTION BROUGHT INTO SOLUTION
		per cent	
Lignin	21.0	64	13.44
Protein	30.2	50	15.10
Hemicelluloses and others	18.8	45	8.46
Total extracted	70.0	....	37.00

These results enable one to compute tentatively that the percentage of carbon in the humus extracted by 4 per cent NaOH solution is about 53. Since this extracted part represents 70 per cent of total humus extracted from the mud, one may conclude from these results that the carbon content of marine humus is about 53 per cent; the factor to be used for calculating the humus content in marine bottoms on the basis of the organic carbon content would, therefore be 1.887. One assumes thereby that the carbon distribution in the 30 per cent of the humus in the mud not extracted by the alkali solution is the same as in the extracted part. That this is not fully the case is brought out by the fact that a much smaller amount of nitrogen than of carbon is left in the residual mud, when compared with the original mud. Whether the residual carbon and nitrogen represent, partly at least, chitinous materials, or other carbohydrate-like complexes, still remains to be determined. Certainly their carbon content should not be higher than is the case of the complexes dissolved by the hot alkali treatment.

An attempt was made to analyze the marine humus also by another procedure developed for the study of soil humus (22). One-hundred-gram portions of the dry mud were extracted for 8 hours with sulfuric ether in Soxhlets.

The ether-soluble portion was determined by evaporating the ether and drying the residue in a weighing bottle. The residual mud was then treated with boiling 95 per cent alcohol, for 10 hours; the alcohol extract was evaporated on a water bath, the residue dried, weighed, and ignited. This was followed by extraction with hot water, on a boiling water bath, for 2 hours, then by boiling for 2 hours with 2 per cent HCl solution. The residue left after the last treatment was washed, dried, and weighed; aliquot portions were then treated in the cold with 80 per cent H_2SO_4, for 2 hours, followed by dilution with 15 volumes of water and boiling under 15 pounds pressure for 30 minutes; the residue was washed, dried, weighed, and analyzed for total carbon and nitrogen. The results of the analyses are given in table 13. The carbon content of the various fractions was calculated, assuming a certain carbon content which is characteristic of the major constituents of the fraction. The theoretical carbon content of the marine humus, as calculated by this system of analysis was 52.8 per cent. One is thus able to account for 5,125 mgm. of the organic matter out of a total of 5,320 mgm. actually present in the mud, by assuming that the carbon content of the humus in the mud (2.82 per cent total carbon) is 53 per cent.

TABLE 13

Chemical composition of humus in marine mud

100 gm. of dry mud containing 2.82 per cent carbon

ORGANIC MATTER FRACTION	YIELD	APPROXIMATE CARBON CONTENT	TOTAL CARBON
	mgm.	*per cent*	*mgm.*
Ether-soluble	57	70	40
Alcohol-soluble organic matter	592	50	296
Hot water-soluble organic matter	389	46	179
Organic matter soluble in 2 per cent HCl*	527*	42	222
Soluble in cold 80 per cent H_2SO_4	919†	42	386
Protein insoluble in cold 80 per cent H_2SO_4	769	50	385
Lignin-like complexes (insoluble in cold 80 per cent H_2SO_4)	1,872	64	1,198
Total	5,125	.....	2,706
Carbon content of organic matter, per cent	.....	.....	52.8

* Calculated from organic carbon in solution.

† Calculated by loss in carbon content on treatment.

The two sets of analyses give a close enough factor for determinations of this type, which involve organic complexes the exact chemical nature of which is not yet understood. One can thus conclude that the carbon content of the humus in marine mud is 53 per cent and that a factor of 1.887 can be recommended for calculating the organic matter of the mud on the basis of its organic carbon content. This factor may, of course, be found to be too low in the case of the humus in deep oceanic sediments and too high in case of older geological deposits.

Origin of marine humus

Any study of the chemical nature of marine humus must also consider, (*a*) the origin of this humus, which results from the decomposition of plant and animal residues in the water and in the marine bottoms, by the action of bacteria, preceded or not by animal digestion, in addition of course, to terrigenous deposition; (*b*) the further decomposition or mineralization of the marine humus, with the result that the important elements carbon, nitrogen, and phosphorus are returned to circulation in the sea, in the form of carbon dioxide, ammonia, and phosphoric acid, and are thus made available again for the growth and photosynthetic activities of the phytoplankton.

The problem of decomposition of marine algae and of zooplankton by bacteria is discussed in detail in another publication (21). Only a small part of the organic matter built up by marine plants passes through the animal body, and the larger part is destroyed through direct bacterial action when the plants die (13). It remains to be determined whether these dead plants are decomposed as a whole, leaving no residues, or whether substances are formed in the process of decomposition which are similar to the humus of land soils. According to Boysen-Jensen (2), Zostera is the chief source of organic matter in the sea bottom, especially in the fjords, and not the plankton; in the deeper and more open waters, however, the plankton organisms were found to be an important source of the organic matter in the sea bottom. The high nitrogen content of the sea bottom suggested the idea that the non-nitrogenous constituents rather than the nitrogenous complexes have undergone decomposition. The latter were believed to be transformed into humic compounds which are less readily destroyed. Jensen actually demonstrated that the older the Zostera plant, the less in the amount of nitrogen which can be made readily soluble by the action of pancreatic enzymes upon it.

The presence of different complexes in the marine humus, especially the α-fraction, which is a ligno-protein complex, and the γ-fraction, which is a carbohydrate-protein complex, points to the possibility that this humus, at least in the case of deposits not far from shore, may be derived from different sources. There is no doubt that the decomposition of Zostera, which contains lignin and cellulose similar to land plants, may lead to the formation of ligno-protein complexes. The fact remains, however, that few other marine plants contain very much lignin. The possibility thus suggests itself that the ligno-protein complexes (α-fraction) found in marine humus are partly of terrestrial origin and may also partly be due to the decomposition of plant remains at shore points; however, other constituents of the marine humus, as in the case of the γ-fraction, may chiefly be of marine origin.

Decomposition of marine humus

There is no doubt that marine humus, like land humus, is resistant to further rapid decomposition by bacteria; otherwise it would not accumulate very extensively in the marine sediments. The possibility exists, however, that this humus is not absolutely resistant, but undergoes a slow but gradual decomposition. To test this, 150-gm. portions of dry marine mud were placed in long-necked flasks, moistened with 50 cc. of tap water or salt water, and connected with an aeration apparatus; CO_2-free air was passed over the mud, then through $N/6$ $Ba(OH)_2$ solution, in order to remove the CO_2 liberated in the process of decomposition. The solution was then titrated with $N/6$ oxalic acid. The muds were incubated at 28°C. for 14 to 25 days. At the end of that period, the mud was removed from the flasks and analyzed for ammonia and nitrate. The moist mud was plated out on suitable media; only bacteria were found, but not fungi or actinomyces, a phenomenon characteristic of the marine mud under natural conditions. For comparison, a similar amount of field soil, known as Sassafrass loam, with a similar amount of organic matter, was allowed to decompose under the same conditions. The results are given in table 14.

TABLE 14

Decomposition of the humus in marine mud, as compared with that of normal soil

MATERIAL USED	150 GM. OF DRY MUD—50 CC. WATER					
	Water used	Incubation	CO_2 liberated	Nitrogen liberated		C/N of products liberated
				NH_3-N	NO_3-N	
		days	*mgm.*	*mgm.*	*mgm.*	
Marine mud	Tap water	25	75.3	13.5	0	5.6
Marine mud	Salt water	14	55.8	11.1	0	5.05
Field soil	Tap water	25	43.7	0.5	4.8	8.2

These results show that the organic matter in the marine mud, which has previously been dried, is able to decompose very actively, more so even than a similar amount of organic matter in soil, as shown by the greater evolution of CO_2. There was a much greater relative liberation of the nitrogen in an available form from the mud than from the soil, as shown by the narrower C:N ratio of these two important elements, liberated in a mineralized state. This may be due partly to the relatively greater nitrogen content of the mud, as well as to the fact that the nitrogenous complexes in the mud humus can be decomposed more rapidly than those of the soil humus. The last assumption is born out by the fact that the carbon-nitrogen ratio of marine humus usually widens with an increase in depth of mud in sediment, as shown in table 5. In the case of land soils, the tendency is for this ratio to become narrower with depth of soil. In the flasks containing the land soil, the ammonia formed in the decomposition of the organic matter by the microorganisms was rapidly changed to nitrate, as a result of the activity of the nitrifying bacteria. In the case of the marine mud, however, all the nitrogen liberated was left as ammonia, because of the fact that no nitrification took place in the mud, under the particular experimental conditions. It is possible that the drying of the mud resulted in the destruction of such organisms.

These results serve to emphasize the fact that the humus in marine mud, when placed under favorable conditions, can decompose readily and liberate the nutrient elements back into circulation. Actually, 150 gm. of mud containing 3,000 mgm. of carbon liberated 55.8 mgm. of carbon as CO_2 in 14 days and 75.3 mgm. in 25 days, under two sets of conditions, while, out of a total of 345 mgm. of nitrogen, 11.1 mgm. and 13.5 mgm. were liberated as ammonia in the corresponding periods. There is no doubt that the drying of the mud was partly, at least, responsible for this comparatively rapid decomposition of the humus; however, even the organic matter in moist mud was found later to decompose but at a slower rate.

Importance of humus in marine processes

It has been suggested (20) that some of the most important processes carried out by bacteria in the sea take place in the sea bottom, whether the latter is made up of mud, sand, or the various marine oozes; these activities take place probably in the very surface layers of the bottom. Among these, one need only mention the decomposition of the numerous residues of plant and animal life in the sea, which sooner or later reach the bottom, and the formation of nitrates, by oxidation of the ammonia first to nitrite and then to nitrate. The relatively greater abundance of bacteria in the sea bottom, especially in the very surface layers, as compared with the numbers in the sea water, is sufficient evidence to the importance of the marine bottom as a seat of bacterial activities in the sea. The abundance of humus in the marine bottom seems to have a very definite influence upon the bacterial numbers; one finds frequently a very close correlation between the abundance of humus and the abundance of bacteria in marine muds, as shown elsewhere (20): the greater the humus content of the mud the greater were the numbers of bacteria found to be within a certain close area of the sea. One need not expect, however, that this correlation will always hold. The physical conditions of the sea bottom, especially the mechanical composition of the inorganic fraction, the colloid content, etc., may influence considerably the abundance of bacteria.

The marine humus thus makes the sea bottom a favorable medium and substrate in which the bacteria grow and upon which they live. The rôle of the marine humus in animal nutrition is still a matter of conjecture; however, there is no doubt that it makes the sea bottom also a more favorable medium for the growth of numerous animals that live in it or upon it.

In addition to the aforementioned function, humus also plays an important part in the sedimentation processes taking place in the sea. It is sufficient to refer here to the preliminary investigations of Raymond and Stetson (8), on the possible significance of a jelly-like organic material on the surface of the sea bottom, as an agent of transportation of sand grains.

SUMMARY

A study has been made of the abundance and distribution of organic matter in the marine bottom, largely mud sediments. A few sand bottoms and deep oceanic deposits were also analyzed.

It is suggested that the organic matter in the marine bottom be designated "marine humus," to make it comparable to the soil humus, found in land soils.

The abundance of humus in the sea bottom was found to vary considerably, depending on the distance from shore, the topography of the sea bottom, the nature of the inorganic residues, and the depth of the water.

The humus content in the marine mud was found to diminish gradually with depth of mud; in some cases, however, the humus content may increase with depth, depending on the nature of the material deposited.

Marine humus is characterized by a more or less constant ratio of carbon to nitrogen, which ranges from 8 to 12; the lower the humus content the wider the ratio.

Marine humus is shown to be very complex in chemical composition; it can be separated into a number of fractions which possess distinctly different properties. Several such fractions were isolated, one of which, termed "α-fraction," consists predominantly of lignin and protein and is similar to a corresponding fraction isolated from land soil, and another, termed "γ-fraction," is largely a hemicellulose-protein complex.

It is suggested that the abundance of organic matter, or marine humus, in the sea bottom be measured by determining the total organic carbon by some convenient method and calculating the humus by the use of the factor 1.887.

Humus is formed in the marine bottom as a result of bacterial decomposition of plant and animal residues of the sea. Close to shore it is partly of terrestrial and partly of marine origin. Marine humus is more resistant to further decomposition than are fresh plant and animal residues, especially at lower depths. In the presence of sufficient oxygen, the humus undergoes a process of slow decomposition, as shown by the liberation of a continuous stream of CO_2 and ammonia.

Humus gives certain characteristic properties to the marine bottom, making it a more favorable medium for the growth of bacteria and animal forms.

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THE LAWS OF SOIL COLLOIDAL BEHAVIOR: XI. ELECTRODIALYSIS IN RELATION TO SOIL PROCESSES[1]

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In 1923 this writer applied for the first time the method of electrodialysis to the removal and determination of the exchangeable cations in the soil and found at the same time a way to obtain a sample of soil in its unsaturated (free ampholytoid) condition without the use of any leaching agent.[3]

The method has since been employed by many workers, and much has been learned about the process, the use of various types of cells, electrodes, and membranes. But in all of this work attention has chiefly been focused upon the exchangeable cations and other plant nutrients. The changes taking place in the soil colloidal complex as a result of its desaturation by electrodialysis have not been investigated although such changes are quite evident from the appearance of silica and sesquioxides in the cathode chamber after the pH of the soil has been reduced to a certain value. The work here presented represents a preliminary investigation of this problem.

In his original work the writer found when electrodialyzing a Sharkey soil colloid that the bulk of the strong bases were removed in the form of a clear solution during the first 12 hours. The following 6 hours yielded a light colored sediment in the cathode solution which then changed to a reddish brown color. At the end of 42 hours the titre and also the quantity of sediment had become very small. The three fractions were analyzed separately with the result shown in table 96.

The release of the cations as a function of the time is shown in figure 38. The position of the curves is in general the same as that obtained by Oden and Löddesöl (6), who give the results of the electrodialysis of three different soils. They separated their electrodialyzates at short intervals of time into a much greater number of fractions and determined in addition the pH of the soil at each interval. The writer determined the pH of the soil only in one instance, namely, at the point where the light colored sediment began to appear, or after 12 hours' dialysis. The pH was then 5.4. This again agrees with the findings of Oden and Löddesöl.

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[3] As a result of an unusual situation this work, although finished in the fall of 1924, could not be published until September, 1926 (3).

The cations of the insoluble bases, Al, Fe, Mg, and also Mn, (comp. table 96) become mobile only after the pH has been reduced to a certain value exactly as under natural conditions. Above these pH values the compounds in which these ions exist in the soil are too slightly dissociated. Under natural humid conditions the soluble strong bases (together with silica which ionizes at high pH) are always removed from the soil by the drainage water whereas Al and Fe become mobile only under conditions of a certain degree of acidity when podzolization sets in.

TABLE 96

Successive fractions of bases removed by electrodialysis of the Sharkey soil colloid

Milliequivalents per 100 gm. colloid

HOURS OF ELECTRO-DIALYSIS	Al_2O_3	Fe_2O_3	CaO	MgO	K_2O	Na_2O	MnO
0–12	trace	trace	38.40	0.35	1.94	0.97	trace
12–18	3.18	0.30	4.89	6.81	0.35	0.29	0.05
18–42	6.42	3.42	2.07	12.25	0.45	0.22	0.25
Total....	9.60	3.72	45.36	19.41	2.74	1.48	0.30

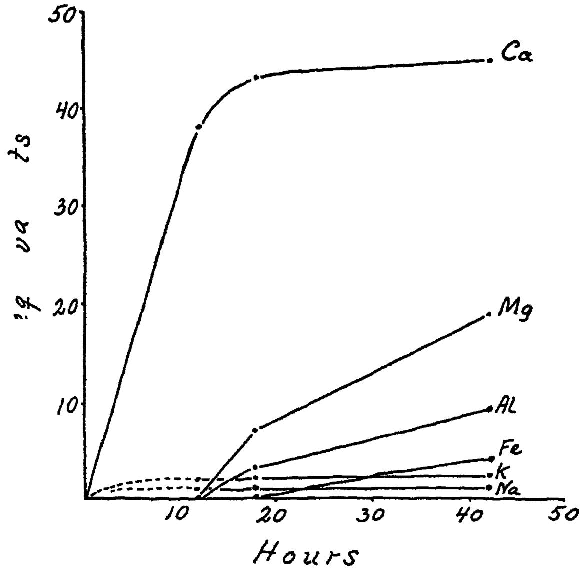

FIG. 38. THE RATE OF EXTRACTION OF THE VARIOUS CATIONS BY ELECTRODIALYSIS OF THE SHARKEY SOIL COLLOID

Electrodialysis of the soil might therefore be looked upon as a highly intensified reproduction of the natural process of leaching. In electrodialysis it is only the ions which are removed but, since compounds are in general more soluble (or dispersible) in the ionized condition, this is also true in the process of leaching. The soil colloidal complex is amphoteric, and which one of the two groups (acid or basic) will become most ionized, and therefore, by hydrolysis, most soluble will depend upon the pH. We shall here present some experimental evidence to show how closely the natural soil processes are reproduced in an electrodialysis cell. (A cell of the type designed by the writer in which the soil is left undisturbed in the central compartment is best suited to bring out the relationship.)

THE MOBILITY OF Al AND Fe

In the first experiments in the electrodialysis of soils it was observed that, although a heavy sediment appeared in the cathode chamber after the bulk of the strong bases had been removed, this sediment later diminished and, in the case of the Sharkey soil colloid, was very slight after 42 hours. Was this because Al and Fe exist, like the cations of the strong bases, in an exchangeable and a "non-exchangeable" condition and that the exchangeable quantity was by this time all removed? This conclusion seemed to be supported by the fact that the Sharkey soil colloid, which, of the two soil colloids investigated, has the highest silica/sesquioxide ratio and the highest exchange capacity, yielded also the greatest quantity of Al and Fe; that is, the colloid having the lowest sesquioxide content yielded most Al and Fe. (A laterite which has since then been electrodialyzed yielded only traces of Al and Fe.)

But then it was observed that the same electrodialyzed Sharkey colloid yielded again large quantities of Al and Fe after being saturated with Ca and again electrodialyzed.

It was further found, as reported in a separate paper (4), that the same renewal of the appearance of Al and Fe resulted from the neutral salt treatment of the unsaturated colloid no matter how often the latter was rendered unsaturated nor by what method, whether by electrodialysis or by treatment with dilute acid. There was apparently no limit to the amount of Al and Fe which could thus be extracted, but here again the yield was always greater in the Sharkey than in the Norfolk colloid. The idea of an exchangeable and a "non-exchangeable" form of Al and Fe had to be given up.

But why does the Norfolk soil colloid and the laterite, which possess a low cation exchange capacity and contain the highest proportion of Al and Fe, yield the smallest amount of these ions both upon electrodialysis and upon treatment of the unsaturated soils with a neutral salt solution, and why is only a limited quantity of Al and Fe removed each time the soil is electrodialyzed?

The answer to these questions is closely related to the amphoteric nature of the soil complex: its isoelectric point, its ultimate pH, and its point of exchange neutrality. After the development of his theory of isoelectric weathering (5) the writer believed that he could satisfactorily account for these phenomena, but experimental evidence would be more desirable than a theoretical explanation because this would not only supply the proof but would at the same time give support to the theory. The following experiments were therefore performed.

EFFECT OF REVERSING THE CURRENT

The electrodialysis cell employed was the so-called Mattson cell described in the catalogue of the Arthur H. Thomas Company except that a platinum instead of a carbon anode was used. The soil compartment was 1 cm. thick, and the distance between the electrodes when placed up against the glass lattice was about 2 cm. The cell was placed in a 110-volt circuit in series with three 100-watt lamps placed in parallel. A cooling coil was placed in each of the electrode compartments.

TABLE 97

The effect of the reversal of the current upon the quantities of silica and sesquioxides removed from the Sharkey soil by electrodialysis

8-HOUR INTERVAL	BASES BY TITRATION	SiO_2	Al_2O_3	Fe_2O_3
	m.e.	*gm.*	*gm.*	*gm.*
1st	25.05	0.0095	trace	None
2nd	2.45	0.0046	trace	None
3rd	1.20	0.0474	0.0466	0.0512
4th	0.12	0.0084	0.0159	0.0104
		Current reversed		
5th	0.10	0.0812	0.1194	0.0864
6th	0.02	0.0232	0.0345	0.0208
		Current reversed		
7th	0.02	0.0871	0.1472	0.0645
8th	0.02	0.0526	0.0794	0.0380
9th	0.02	0.0325	0.0284	0.0176

A quantity of 83.5 gm. (air-dry basis) of Sharkey clay soil was placed in the cell and electrodialyzed. The cathode dialyzate was separated into fractions at 8-hour intervals. During the first 8-hour interval the solution was drawn off three times but later this was done only at the end of each interval.

During the third interval the sediment was very heavy, indicating much Al and Fe, whereas the following 8 hours yielded only a moderate sediment. At this point the electrodes were exchanged and the current reversed. This produced the effect which had been expected; the sediment became once more very heavy only to decrease again during the second run after the reversal. Again the electrodes were exchanged and the current reversed, and again there was a very heavy sediment, which decreased as before during a second and a third run.

The various fractions were then analyzed for SiO_2, Al_2O_3, and Fe_2O_3. The results are given in table 97, which in addition shows the quantities of titrable bases in each fraction of the cathode dialyzates.

The reversal of the current results in a greatly increased yield of sesquioxides as well as silica. One might at once come to the conclusion that the cathode membrane becomes physically impermeable to the ions through the mineral deposit which forms there. But such a conclusion would lead us away from the solution of the real problem, which is more complex. The fact is that not only the membrane but also the soil next to the cathode membrane become impermeable to the ions, not because of a reduction in the size of the pores, but because of the precipitation of the amphoteric ions at a pH near to that of their isoelectric point. There is formed, on and near the membrane, a layer which corresponds to the B horizon in a podzol profile through which, when fully developed, no ions or ion complexes of Al and Fe can pass. This layer, which is originally formed by the influence of the high pH in the cathode chamber, will ultimately become so enriched in its basoid constituents as to maintain a high enough pH, even at complete unsaturation, to precipitate these ions.

The electrodialysis of a soil results in a pH gradient within the soil mass from the cathode to the anode membranes. The unsaturation of the soil becomes at first greatest next to the anode membrane. As the pH here is reduced to a certain value, Al and later Fe begin to ionize and to move toward the cathode not as single trivalent ions Al^{+++} and Fe^{+++} (the pH is too high for them to exist) but as complex ions such as $Al^{+}(OH)\ (SiO_3H)$, $Al^{++}(SiO_3H)$, or any low multiple of these. This accounts satisfactorily for the appearance of silica in the cathode chamber. The ions will, however, not migrate directly to the cathode with the speed of the common ions, but will repeatedly be precipitated in zones of higher pH where they will become associated with OH ions and lose their charge only again to become ionized as the pH progressively drops through the soil mass in the direction of the cathode. As the "wave" reaches the cathode membrane some of the ions will emerge into the chamber where, by precipitation, they produce the aforementioned sediment whereas others of the ions will precipitate at the membrane itself.

ANALOGY BETWEEN ELECTRODIALYSIS AND PODZOLIZATION

The precipitation of the ions at the membrane builds up a new complex which is richer in sesquioxides and poorer in silica (and humus) than the original soil complex. Its ultimate pH and isoelectric point are therefore higher and will finally reach a value at which no ions of Al and Fe will be able to penetrate the layer, not even after complete unsaturation. No sediment will then appear in the cathode solution but basic material will continue to be ionized within the more acid soil mass and be transported toward the cathode membrane where the amphoteric ions will lose their charge and be precipitated. The colloidal complex near the anode will become more and more acidic while the complex on the cathode side becomes more basic, which is analogous to the development of the A and B horizons in the podzol profile. The pH differs therefore greatly in the two parts of the soil upon the completion of electrodialysis, as will be shown later.

In the preceding experiment the yield of Al and Fe was increased upon each reversal of the current because these ions could retain their charge and be transported through a soil medium which before the reversal had become very acid (while facing the anode membrane).

In order to show that it actually is the building up of an amphoteric layer having a high isoelectric point and therefore a high ultimate pH which causes the precipitation and accumulation of the sesquioxides in the layer adjacent to the cathode membrane (a kind of autocatalytic precipitation) the following experiment was performed.

A fourth compartment 0.5 cm. thick was made between the soil and the cathode compartments. This was filled with aluminum "hydroxide C.P." (Mercks) and electrodialyzed until free from all diffusible ions. The soil compartment was then filled with Sharkey soil (83.5 gm.) and electrodialyzed as in the preceding experiment. All cations would have to pass through the 0.5-cm. layer of $Al(OH)_3$ before reaching the cathode compartment. The $Al(OH)_3$ constituted an amphoteric layer having a high isoelectric point (about pH 8). It represented, so to speak, an artificially interposed B horizon.

The electrodialysis required a long time because of the high resistance of the $Al(OH)_3$ but after 163 hours it was practically complete. The titrable bases amounted then to 23.92 m.e. The cathode solution remained clear throughout the experiment. Magnesium was present in very small quantities but Al_2O_3, Fe_2O_3, and SiO_2 were absent, except for traces so small that they might be ascribed to the glassware and the reagents.

On opening the cell a heavy brownish, mottled crust was found on the parchment between the soil and the $Al(OH)_3$. The soil next to this membrane had a brownish color and the otherwise snow-white hydroxide was pitted with a brownish coloration on the side facing the same parchment.

The $Al(OH)_3$ had effectually prevented the passage of the alumino and ferric silicate cations.

The pH was then determined at three points in the cell and found to be as follows:

Soil next to the anode membrane	pH 3.4
Soil next to the cathode membrane	pH 6.0
Aluminum hydroxide	pH 8.4

The "podzolization" is therefore in evidence by the difference both in color and in pH of the soil at the two sides of the compartment. The whole soil must have been unsaturated but, because of the enrichment of the basoid constituent in the complex at the cathode side, its isoelectric point and therefore its ultimate pH were increased.

EFFECT OF MAINTAINING A HIGH ALKALINITY IN THE CATHODE CHAMBER

The quantities of silica and sesquioxides transported to the cathode solution depend upon the reaction of this solution irrespective of the degree of unsaturation of the soil. This is shown by the following experiment.

Forty grams of electrodialyzed Sharkey soil was saturated with 16 m.e. NaOH and electrodialyzed. After 2 hours, 16.2 m.e. base were found by titration in the cathode solution, which was quite clear. The cathode compartment was refilled with a solution containing 16 m.e. NaOH instead of distilled water, as is usually done, and the electrodialysis was continued for 20 hours.

The *cathode solution* was, at the end of this period, almost clear and contained only 0.0195 gm. SiO_2 and 0.0106 gm. $Al_2O_3 + Fe_2O_3$.

The *anode solution* was clear, faintly yellow, and contained 0.0132 gm. silica.

The cathode membrane showed a thick crusty deposit of a variegated coloration from brown to yellowish brown and mottled with white specks.

The high alkalinity in the cathode solution precipitates the sesquioxides and silica before they emerge into this compartment. *It is obvious that an amphoteric ion cannot be transported across the zone of its isoelectric pH.* This is one of the fundamental principles in the theory of isoelectric weathering (5, IX). The amount of these materials obtained in the cathode solution will therefore depend on how often the solution is withdrawn and replaced by water. The continuous withdrawal of the dialyzates as accomplished by Oden and Wijkström (7) will therefore yield larger quantities and cause a more nearly continuous flow of Al, Fe, and Si.

As to the end point, that is another question. We are continually breaking down the secondary (weathered) soil complex as well as the particles of the original rock minerals. The difference between the exchangeable and the "non-exchangeable" ions is only a difference of degree. The common cations on the surface of the particles are easily exchangeable because of their high degree of dissociation. Al and Fe are displaced by H, and the silicate ion by OH (PO_4 and humate ions, etc.). The displacement of the latter ions is not always apparent because of their failure to ionize and thus become soluble except at a very low or a very high pH. But the makeup of the complex must be different at each pH and in each new ionic environment. The combination between the acidoid and basoid groups is a maximum at the isoelectric point only. We cannot unsaturate a soil, by electrodialysis or otherwise, without altering the constitution of the colloidal complex. In the electrodialysis of a soil for the purpose of obtaining a base unsaturated product the treatment should never be carried too far.

EFFECT OF KEEPING THE SOIL IN A SATURATED CONDITION

If the soil is kept saturated while it is being electrodialyzed by the addition of alkali there is no transport of Al and Fe in either direction, but considerable quantities of SiO_2 and humus appear in the anode solution, as shown by the following experiment.

Forty grams of Na-saturated Sharkey soil was electrodialyzed for 20 hours. At the end of each half hour 5 m.e. of NaOH were added to the anode solution.

In this way the soil was prevented from becoming unsaturated. The cathode solution, which was quite clear and colorless, was withdrawn every few hours.

At the end of 20 hours the anode solution was clear but of a brown color, indicating considerable humus. The analysis showed the following:

SiO_2 in anode solution	0.2180 gm.
SiO_2 in cathode solution	0.0014 gm.
R_2O_3 in cathode solution	trace

The cathode membrane was entirely free from any stain or deposit. There was therefore no movement of Al and Fe.

The experiment shows also that silica and humus migrate anodically in the alkali-saturated soil. The fact that very little silica and humus were removed where the saturation of the soil was not maintained indicates that as soon as the pH of the soil is reduced to a certain value the dispersibility of these anodic materials becomes too low for a passage through the membrane. This is, of course, a direct result of their failure to ionize sufficiently at the lower pH.

LATERIZATION

The liberation and anodic transport of silica in the soil at high pH and the liberation and cathodic transport of aluminum and iron at low pH are in complete agreement with the theory of isoelectric weathering and have their counterpart in nature in laterization in the first case and podzolization in the latter. It is obvious that if the last experiment had been continued indefinitely there would finally have resulted a lateritic soil, whereas in the preceding experiments the characteristics of a podzol were developed.

Since the accumulation of sesquioxides at the cathode membrane prevents the passage of ionic Al and Fe, we can now readily understand why soils rich in sesquioxides and poor in silica, such as the Norfolk and Sassafras soils and especially the laterites, yield much less Al and Fe when electrodialyzed or when treated with a neutral salt solution in their unsaturated condition. The isoelectric point of these soils is higher and their ultimate pH and their pH of exchange neutrality are therefore also higher as compared to soils of the Sharkey type. The Al and Fe, being less ionized, are less transported by the electric current and less dissolved by the exchange acidity, which must always be low in soils of this type.

By lowering their isoelectric point by the addition of humus or some other acidoid, the mobility of Al and Fe may be greatly increased, as the following experiment shows.

EFFECT OF LOWERING THE ISOELECTRIC POINT

A sample of soil was collected from the B horizon of the Sassafras loam at Colonial Gardens, near New Brunswick. The soil was compact and of a reddish brown color. A 100-gm. sample of the soil was electrodialyzed in its natural condition, and another 100-gm. sample was first mixed in the form of a suspension with 6 gm. electrodialyzed humic acid (5, IV). The electrodialysis was continued for 45 hours. The results are shown in table 98.

The humus caused the ultimate pH to drop from 5.15 to 3.6 and increased greatly the mobility of Al and Fe (also the associated silica). The podzolizing effect of unsaturated humic acids is therefore most strikingly shown by this experiment. The original complex must be isoelectric at a pH about 5.0. In the unsaturated condition the pH of the soil can never fall below this value. The ionization and mobility of Al and Fe must therefore remain very limited. Upon the addition of humic acid a new complex having a lower isoelectric point is formed. The ultimate pH is therefore also lowered. At the greater acidity, Al and Fe become more ionized and are liberated by hydrolytic cleavage forming complex cations carrying silicate, humate, and other anions of weak acids.

TABLE 98

Electrodialysis of a Sassafras loam (B horizon sample) with and without the addition of humic acid

	NO HUMIC ACID	6 GM. HUMIC ACID IN 100 GM. SOIL
	Alkalinity of cathode solution	
hours	*m.e.*	*m.e.*
1–3	3.3	4.5
3–21	0.4	0.2
21–45	0.2	0.05
Total..................	3.9	4.75
	Sesquioxides and silica in cathode solution	
	gm.	*gm.*
SiO_2	0.0039	0.0660
Al_2O_3	0.0074	0.0969
Fe_2O_3	trace	0.0504
	pH after electrodialysis (ultimate pH)	
	5.15	3.6

It should be pointed out that although an amphoteric colloid is most stable at the isoelectric point a compound might even at this point be quite soluble. Isoelectric silicates, phosphates, and humates of Al and Fe containing a high proportion of the acid radicals and therefore isoelectric at low pH always precipitated in equilibrium with a certain concentration of the constituents in the solution (5, III). Amphoteric compounds of this type undergo complete hydrolysis at very high and at very low pH values. If the products of this hydrolysis are molecularly soluble the whole colloid will dissolve, if not, then only that constituent which is ionized will dissolve, namely, the basic group at low pH and the acid group at high pH.

THE MOBILITY OF Mg

From figure 38 and from the work of Oden and Löddesöl it is evident that Mg becomes mobile only after the bulk of the other cations of the strong bases, Ca, K, and Na, have been removed. Is this because Mg ionizes, like Al and Fe, only at a lower pH or is it merely because Ca, K, and Na are more easily ionized and are therefore the first to be transported by the current?

Magnesium is displaceable from a neutral soil by the neutral salt treatment. Al and Fe are not. But Wiegner and Jenny (9, 1) have made the important observation that whereas the displacing power (eintausch) of the divalent cations places them in the normal lyotropic order:

$$\mathrm{Mg} < \mathrm{Ca} < \mathrm{Ba}$$

the ease with which these cations are displaced when once in combination with the permutite complex (austausch) is the same instead of the reverse, that is, Mg is the most difficult to displace. Wiegner ascribes this anomaly to the solubility of the hydroxides, which is

$$\mathrm{Ba(OH)_2} > \mathrm{Ca(OH)_2} > \mathrm{Mg(OH)_2}$$

Wiegner and Jenny claim that the inner layer of anions consists partly of OH ions, which bind the Mg ions most firmly. This makes the colloidal complex a mere carrier (adsorbent) of anions and cations, which together constitute the double layer. The author does not wish to deny this possibility. Consider for example a crystal of silver chloride $(\mathrm{Ag\ Cl})_x$ which becomes negatively charged by NaCl thus:

$$[(\mathrm{Ag\ Cl})_x\ \mathrm{Cl}]^-\quad \mathrm{Na}^+$$

or positively charged by $\mathrm{AgNO_3}$ thus:

$$[(\mathrm{Ag\ Cl})_x\ \mathrm{Ag}]^+\quad \mathrm{NO_3}^-$$

Similarly $\mathrm{Al(OH)_3}$ forms a negative ion in the presence of alkalies. This is probably an addition compound, as represented by the formula

$$[(\mathrm{Al(OH)_3})^2]_x\cdot\mathrm{Al(OH)_4}^-\quad \mathrm{Na}^+,$$

which at high pH reaches the ultimate dispersion and becomes

$$\mathrm{Al(OH)_4}^-\quad \mathrm{Na}^+$$

Or consider the KCN "adsorption" by AgCN. The association of Ag^+ and CN^- is so strong that the silver ion ultimately binds 2 CN^- ions. The ionic density or charge becomes so great that the complex

$$[(\mathrm{AgCN})_x\cdot\mathrm{Ag(CN)_2}]^-\quad \mathrm{K}^+$$

in the presence of sufficient cyanide disperses into the single ionic species.

$$\mathrm{Ag(CN)_2}^- + \mathrm{K}^+$$

When the "colloid" becomes a stoichiometric, "true electrolyte," the *chemistry* of the adsorption becomes perhaps more apparent and acceptable. In the realm of solution chemistry we find a number of similar addition (adsorption) compounds in which both of a pair of ions add themselves to another molecular species. That both of the ions must be adsorbed together, except when the diffusible ion becomes displaced by another, is quite obvious.

This all illustrates how a double layer may be formed by the "adsorption" of both of a pair of free diffusible ions. The adsorption theory of Wiegner, therefore, does not fundamentally differ from the theory here adopted that the soil colloids are themselves ionogens and assume their charge or inner electrical layer by dissociation of diffusible ions, provided we do not fall into the error of defining adsorption as "physical" or "colloidal" as distinguished from chemical reaction. (In the last analysis chemistry is, of course, nothing but the physics of ions and molecules.)

But Wiegner's and Jenny's explanation is not sufficient to account for the anomaly. The sesquioxides which do combine with (adsorb) bases do so only at high pH values, that is, above their isoelectric point. On the acid side of the neutral point they are electropositive and combine with, and exchange, no cations. We have, however, seen that the introduction of silica, or other acidoid, into the complex renders it exchange active far over on the acid side. The bond between the exchangeable cation and the colloidal complex must therefore be through the silicate (or other acidoid) group. The anomaly might therefore be linked to the properties of the various silicates of the divalent cations. We shall here find a complete agreement, but, before the results of this investigation are shown, the following experiment on the electrodialysis of the Mg-saturated Sharkey soil will be given.

Two 40-gm. samples of the electrodialyzed Sharkey soil were each treated with 10.66 m.e. of MgO with some water on the water bath. The samples were then electrodialyzed, with the difference that, in one of the cells, a solution containing 10 m.e. of NaOH was added to the cathode compartment instead of distilled water so as to maintain a strongly alkaline reaction. The results are shown in table 99.

Although a high alkalinity prevents the appearance of Al and Fe in the cathode solution, it has no effect upon the mobility of Mg. The fact that the mobility of Mg appears to be greater where NaOH was added to the cathode solution is to be ascribed to a greater conductivity of the solution, thus establishing a higher P.D. within the soil mass.

We note also that when the soil is saturated with Mg alone there is no lag in its mobility as when Ca, K, and Na are also present. The yield of Mg is high at the outset and soon falls off to a low value. The remarkable thing is that only slightly more than one half of the added magnesium is easily removable by electrodialysis. The remaining fraction appears to be fixed in the soil. Löddesöl reports the same observation (2).

We may therefore conclude that the lag usually observed in the mobility of

Mg and the difficulty with which it is displaced by neutral salt cations are due to the formation of compounds which, at least in part, are more stable than those of the other cations. This view is also supported by the relatively high content of "non-exchangeable" Mg in the natural colloids. Poluinov found in the B horizon of certain soils a Mg mineral which he named "polygorshite" (8).

When the products of weathering unite to form crystalline compounds it is obvious that those combinations which are least ionized and therefore most stable will be the ones first to be formed and the last to be decomposed. Where the leaching is limited, even comparatively unstable compounds may be formed and persist for a long time. Thus in young soils and in soils of the arid and semi-raid regions we find strongly electronegative soil colloids high in bases (notably Mg) and silica, such as those of the montmorillonite type. Since under conditions of a high pH the strong bases and the silica are more soluble (ionized) than the sesquioxide, it is obvious that such compounds could not long persist in a region of high rainfall. An isoelectric weathering would here dominate.

TABLE 99

Electrodialysis of Mg-saturated Sharkey soil with and without NaOH in the cathode compartment

TIME	NO NaOH ADDED TO CATHODE COMPARTMENT	NaOH ADDED TO CATHODE COMPARTMENT
	Mg. found in cathode solution	
hours	*m.e.*	*m.e.*
1½	4.02	4.97
2	0.98	0.40
16	0.61	0.21
Total..................	5.61	5.58
	Al and Fe present	No Al and Fe

THE SILICATES OF Mg, Ca, AND Ba

Five millimols of Na-silicate were added to each of three beakers. Two and one-half milliequivalents of $MgCl_2$, $CaCl_2$, and $BaCl_2$ were then added separately to the silicate solutions. The precipitates were transferred to the filter and leached with neutral, *N* NH_4Cl solution. The filtrate from each was separated into three 100-cc. fractions. By this amount of leaching all of the exchangeable cations should have been displaced if the material had consisted of soil colloids. Each of the precipitates was now washed with 100-cc. 2*N* HCl. The quantities of Mg, Ca, and Ba removed in the different fractions are shown in table 100.

This displaceability is

$$Ba > Ca > Mg$$

just as it was found by Wiegner and Jenny in the permutite complex. The Mg is so firmly held by the silicate radical that a strong acid solution is required for its complete displacement. In one experiment performed several years ago the author tried to displace all the Mg from its silicate by NH_4Cl, but after the precipitate had been washed with 1,200 cc. the filtrate still showed the presence of Mg.

It would be interesting to determine the displaceability of the various cations from other compounds such as the humates and phosphates of Al and Fe.

Since now Mg forms the most stable combination with the silicate group and is therefore displaced with the greatest difficulty, why then is it that, as a displacing ion, it is the weakest? Is this because Mg is most highly hydrated as an ion and has therefore a lower self-potential and is less attracted by the charge of opposite sign of the colloidal particle? We might assume that the colloidal particle undergoes, because of its highly multivalent character, a very limited dissociation. The critical potential, which is higher the more highly the dissociated diffusible ions are hydrated, is attained at a very moderate degree of dissociation. Even the low degree of dissociation of the Mg ions might be more than sufficient to attain this critical value.

TABLE 100

The displaceability of Mg, Ca, and Ba from the respective silicates by NH_4Cl

SILICATE AND ION EXTRACTED	NH_4Cl EXTRACTS			2 *N* HC EXTRACT, 100 cc.
	1st 100 cc.	2nd 100 cc.	3rd 100 cc.	
	m.e.	*m.e.*	*m.e.*	*m.e.*
Mg	0.92	0.22	0.12	1.23
Ca	2.25	0.19	0.06	None
Ba	2.47	0.05	trace	None

Ca and Ba were found by the Donnan distribution (5, I) to be less dissociated than Mg by the soil complex. The Mg-saturated complex imbibes more water, and Mg is the weakest flocculating ion. This points to a greater dissociation of this ion. The Ca and Ba ions might be less dissociated by the complex because of their lower hydration and higher self-potential in spite of the fact that these ions are actually less firmly associated and therefore more easily displaced in the presence of electrolytes which suppress the potential or activity of all ions. In the presence of electrolytes the potential or activity of the highly multivalent colloidal ion is greatly suppressed, far below its critical value. The displaceability will then be governed by the dissociability for then no electrostatic attraction prevents the slightly hydrated ions from dissociating. The displacing power will, however, still depend on the hydration of the ions.

The Mg ion would then possess a weaker displacing power (probably only in moderate concentrations) because, associated with many water molecules which partially suppress its charge, it is less strongly attracted by the opposite charge on the complex. It would be the most difficult ion to displace because once in combination with the silicate group it remains most firmly associated.

This reasoning would, of course, not apply to ordinary simple electrolytes which may undergo a complete dissociation and yet not exceed the critical potential, but colloidal ionogens, whose valence runs into the hundreds of thousands, dissociate, because of electrostatic forces, to a very limited extent. For this reason it is here not the ease with which the complex dissociates but rather the nature of the diffusible ion (its volume, hydration, valence, and potential) which determines the degree of dissociation. This accounts for the Hofmeister series in colloid chemistry.

SUMMARY

The electrodialysis of a soil does not end with the removal of the exchangeable cations. After a certain degree of unsaturation has taken place Al and Fe (together with some associated silica) move toward the cathode, at first emerging into the cathode compartment (if the alkalinity there is not too high) and later building up a layer next to the cathode membrane which corresponds to the B horizon of the podzol profile. This layer becomes enriched in sesquioxides and prevents finally, by virtue of its high ultimate pH, the passage of Al and Fe completely.

If the polarity of the cell is reversed the sesquioxides reappear again in the cathode compartment until a new impermeable layer has been formed on the other side. This process can be repeated indefinitely.

When a layer of $Al(OH)_3$ is interposed in a fourth compartment, no trace of Al and Fe will reach the cathode compartment.

The process is identical to podzolization and can be explained on the basis of the theory of isoelectric weathering.

If the soil is maintained in a saturated condition by the continuous addition of NaOH to the anode compartment, silica and humus will move to the anode whereas Al and Fe show no mobility in either direction.

This process is identical to laterization.

At low pH Al and Fe ionize and become mobile. At high pH silica and humus are ionized and become mobile. The strong bases are mobile at all pH values.

The mobility of Mg has also been studied. The low displaceability of Mg is due to the stability of the silicate. The displaceability of the divalent cations from their silicates by NH_4Cl is

$$Ba > Ca > Mg$$

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CONCERNING THE LENGTH OF INCUBATION PERIOD IN PHYSIOLOGICAL STUDIES OF BACTERIA[1]

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The research student in perusing the literature relative to physiological studies of soil bacteria must soon become amazed at the variations in methods employed by soil biologists. Seldom does he find two laboratories employing identical methods in a study of the same phenomenon. There are no recognized "standard methods" and probably none should be so designated, at least until a particular method can be shown to be superior to others. Similar conditions will, of course, be found to have existed in the early development of any science. And yet it would seem that where results obtained in one laboratory are to be compared with those obtained in another, the technic employed in the two should be so similar that one could be reasonably certain results were not materially influenced by the methods employed. This has not been universally true in the field of soil microbiology.

Of the laboratory procedures subject to wide variation none is more evident, at least in certain types of experiments, than the length of the incubation period. It is true that in many laboratories of recent years the tendency in the study of physiological phenomena has been to employ a prolonged incubation period and to measure the metabolic products at more or less definite intervals, thereby obtaining data from which tendency curves for the particular phenomenon may be constructed. This method perhaps approaches the ideal more nearly than does any other. Unfortunately, there are many lines of research where, because of the large number of comparisons to be made, such a method is obviously impracticable. This is particularly true in the survey type of research. Under such conditions the investigator is forced to adopt some definite period of time for the incubation of cultures, and too frequently the time is chosen arbitrarily or selected because some other investigator happened to use it. Observations in this laboratory have led to the conclusion that in some instances this arbitrary method of selecting the incubation period may result in completely defeating the primary objective of the experiments.

For example, in a study of the relative nitrogen fixing ability of the Azotobacter floras of a large number of soils, it was noted that even though very marked differences were evident in the numbers of Azotobacter present, negligible differences were noted in the quantities of nitrogen fixed when measured by the plate method using an arbitrary 14-day incubation period. Studies were undertaken to ascertain the cause of this anomalous condition. Description of three experiments will be sufficient to call attention to the point in question.

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TABLE 1

Growth of Azotobacter and fixation of nitrogen from different soils—incubation period varied

SOIL NUMBER	INCUBATION PERIOD												
	2 days	3 days		5 days		7 days		10 days		14 days		21 days	
	Growth*	Growth‡	N fixed	Growth‡	N fixed	Growth‡	N fixed	Growth‡	N fixed	Growth‡	N fixed	Growth‡	N fixed
			mgm.		*mgm.*		*mgm.*		*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
8P	145	75 per cent	3.44	88 per cent	3.75	85 per cent	3.79	90 per cent	3.45	90	3.60	85	3.60
10P	82	50 per cent	2.44	78 per cent	3.98	83 per cent	3.94	90 per cent	3.79	90	3.53	85	3.27
14P	70	78	1.99	83 per cent	4.12	83 per cent	4.01	90 per cent	4.09	90	3.63	90	3.45
17G†	0	0	0.08	2	−0.04	3	1.08	50 per cent	2.81	16	1.23	50	3.15
19P†	0	1	0.03	0.5	−0.04	0.5	0.29	0	−0.31	25	1.16	15	1.27
19G	0	0	−0.07	5	0.04	11	0.75	6	0.34	10	1.24	15	2.21
40G	77	63 per cent	3.23	78 per cent	4.16	75 per cent	4.23	90 per cent	3.93	83	3.63	85	2.92
A	0	0	0.37	0	0.34	0	0.34	0	0.22	0	0.19	0	0.37
C	34	39	1.73	75 per cent	3.60	63 per cent	3.56	80 per cent	3.22	85	3.19	80	3.52

* Number of colonies per plate.

† Number of colonies so small that duplicates did not agree.

‡ Percentage figures indicate proportion of the surface covered by Azotobacter growth; other figures indicate number of colonies per plate.

EXPERIMENTS

Observations had indicated that possibly the length of the incubation period might have something to do with the relative quantities of nitrogen fixed by different soils; accordingly this factor was studied.

Experiment I. Nine soils, known to contain Azotobacter in numbers varying from none to many, were selected for comparison. The nitrogen fixing ability of their Azotobacter floras was determined during incubation periods varying from 3 days to 3 weeks. Fourteen mannite agar plates, prepared from thoroughly washed agar agar, were inoculated with 1 gm. each of the nine soils respectively. Duplicates were analyzed for total nitrogen after 0, 3, 5, 7, 10, 14, and 21 days incubation, the quantities of nitrogen fixed being taken as the differences between the results of analysis after 0 days incubation and subsequent periods. After 2 days incubation and prior to each analysis, an estimate of the relative growth of Azotobacter was made. Colonies were counted, if the number was relatively small or if there was little tendency for colonies to coalesce. Otherwise, an estimate was made of the percentage of the surface covered by Azotobacter growth. This could not be done very accurately, especially for the longer periods of incubation, since there was a tendency for the outlines of the colonies to be obliterated by the growth of molds and the drying out of the mass of Azotobacter. The data obtained are recorded in table 1.

A study of these data will reveal several interesting points, the most important one being the very striking decrease in differences exhibited in the quantities of nitrogen fixed by different soils as the period of incubation increased. At the 3-day period significant differences, more or less correlated with the number of colonies or mass of growth, were evident between any two soils save those in which no Azotobacter growth had taken place, and consequently no fixation of nitrogen had occurred. However, these differences had practically disappeared at the 5-day analysis, never to reappear. After 21 days incubation those soils which contained so few Azotobacter that no fixation of nitrogen had taken place within 3 days were showing nitrogen fixation approaching that in soils with the highest Azotobacter contents. Soil A contained no Azotobacter hence exhibited no fixation, the apparent fixation probably being due to too low values for the control.

Another point worthy of note is that with appreciable numbers of Azotobacter present in a soil the maximum quantities of nitrogen fixed were reached by the end of 5 days incubation, whereas with very few Azotobacter fixation continued up to the twenty-first day. There is also some evidence of the loss of nitrogen after the maximum is reached. From these data it is quite evident that marked differences may be detected in the relative nitrogen fixing abilities of soils when measured by this method if the cultures are incubated for one period of time, whereas slight or no differences may be noted if another incubation period is employed.

Experiment II. In an effort to obtain soils with definite and known variations in Azotobacter content, to make comparisons similar to those recorded in experiment I, two soils, E and C, containing relatively large numbers of Azotobacter, were diluted in varying proportions with soil A known to be free of Azotobacter, and the nitrogen fixing abilities of the various dilutions were then compared as in the preceding experiment. The dilutions prepared were 0, 10, 100, and 1,000, and the incubation periods employed were 0, 3, 5, 7, 14, and 21 days. The quantities of nitrogen fixed, together with the estimates of the growth, are recorded in table 2.

Again for the shorter periods of incubation the quantities of nitrogen fixed are proportional to the known number of Azotobacter present. For the longer periods of incubation in the case of soil E, with a hundred-fold difference in the number of Azotobacter, these differences have practically disappeared. Even with a thousand-fold difference in the number of Azotobacter, the quantities of nitrogen fixed are approaching the same after 21 days. In the case of soil C very marked differences evident in the quantities of nitrogen fixed between the undiluted soil and the dilution of 10 for the shorter periods of incubation have disappeared for the longer periods. The 100 and 1,000 dilutions contained so few Azotobacter that consistent results could not be obtained.

TABLE 2

Growth and fixation of nitrogen by dilutions of soils E and C—incubation period varied

SOIL NUMBER	INCUBATION PERIOD									
	3 days		5 days		7 days		14 days		21 days	
	Growth*	N fixed	Growth*	N fixed	Growth*	N fixed	Growth*	N fixed	Growth*	N fixed
		mgm.		*mgm.*		*mgm.*		*mgm.*		*mgm.*
E	42 per cent	2.66	75 per cent	4.20	90 per cent	4.55	90 per cent	4.06	?†	4.13
E/10	76	0.74	43	2.31	65 per cent	3.71	90 per cent	3.75	?	3.64
E/100	7	−0.14	10	0.53	17	1.33	40	3.05	50 per cent	3.47
E/1000‡	1	−0.07	0	0.07	3	0.42	2	1.40	62 per cent	2.35
C	62	0.49	25 per cent	2.84	65 per cent	3.99	75 per cent	3.92	?	3.57
C/10	6	0	8	0.21	10	1.40	40 per cent	3.50	55 per cent	3.50
C/100‡	0.5	−0.07	1	−0.14	0.5	−0.07	10 per cent	1.19	?	0.53
C/1000‡	0	−0.21	0	−0.21	0.5	−0.11	0.5	0.25	?	−0.11

* Percentage figures indicate proportion of the surface covered by Azotobacter growth; other figures indicate number of colonies per plate.

† Impossible to estimate accurately.

‡ Number of colonies so small that duplicates did not agree.

From these data it is again evident that differences in the physiological activity between two soils with a ten-fold difference in the number of organisms can easily be detected if the incubation period is relatively short, i.e., 3 to 5 days, whereas such differences may not be evident with a hundred-fold or even a thousand-fold difference in the number of organisms if the incubation period is prolonged.

Experiment III. Evidence that the conditions noted in the two preceding experiments, conducted with naturally occurring mixed soil cultures, would obtain with pure cultures is furnished by the data submitted in table 3. Some information as to the relative effect of the number of Azotobacter cells as compared with the number of points of inoculation or colonies is also furnished by these data.

In this experiment a pure culture of Azotobacter grown on washed agar was suspended in saline, and dilutions of 0, 16, 64, and 1,000 were prepared. Washed agar plates were then inoculated with equal quantities of the various dilutions at 4, 16, and 64 points approximately equal distance from one another. These were then incubated for 3-, 7-, and 21-day periods, after which they were analyzed for total nitrogen. The relative numbers of organisms therefore varied from 1 to 16,000 per plate while the number of colonies varied from 4 to 64.

TABLE 3

Fixation of nitrogen by various dilutions of a pure culture of Azotobacter—incubation period varied

SAMPLE NUMBER	DILUTION	POINTS OF INOCULATION	RELATIVE NUMBER OF AZOTOBACTER	NITROGEN FIXED		
				3 days	7 days	21 days
				mgm.	*mgm.*	*mgm.*
1	0	4	1,000.0	0.67	0.67	1.44
2	0	16	4,000.0	0.81	1.30	1.79
3	0	64	16,000.0	1.44	1.37	1.72
4	16	4	62.5	0.32	0.67	1.44
5	16	16	250.0	0.91	1.30	1.23
6	16	64	1,000.0	1.65	1.54	1.68
7	64	4	15.6	0.25	0.60	1.44
8	64	16	62.5	0.81	1.37	1.51
9	64	64	250.0	1.58	1.58	1.79
10	1,000	4	1.0	0.18	0.18	1.40
11	1,000	16	4.0	0.53	1.23	1.44
12	1,000	64	16.0	1.16	1.72	2.07

The data presented in table 3 show that the number of points of inoculation is of far more significance in determining the rate of nitrogen fixation than is the number of organisms. During the 3-day incubation period there was a direct relationship between the number of points of inoculation and the quantity of nitrogen fixed for each dilution. Since the number of organisms of any given dilution introduced into the plates varied directly with the number of points of innoculation it might be contended that the number of organisms was just as important as the number of points of inoculation. However, if comparisons are made between different dilutions, variations in the number of organisms with constant numbers of colonies as well as variations in the number of colonies with constant numbers of organisms can be observed. For example, samples 1 and 6, 4 and 8, 5 and 9, and 7 and 12 contained in each instance approximately the same number of organisms, yet the quantities of nitrogen fixed for the 3- and 7-day periods were in every comparison greater for the larger number of points of inoculation. On the other hand the numbers of colonies were constant in 1, 4, 7, and 10; 2, 5, 8, and 11; and 3, 6, 9, and 12. Although there are differences in favor of the larger number of organisms, particularly for the shortest period of incubation and the smallest number of colonies, such differences in general are not nearly so marked as the differences due to variations in the number of colonies, even though the latter varied only sixteen-fold whereas the former varied a thousand-fold. Again, marked differences in the quantities of nitrogen fixed, corresponding with known differences in the bacterial content, that were evident after 3 or even 5 days incubation have practically disappeared after 21 days.

DISCUSSION

Failure to detect differences in the nitrogen fixing ability of different soils, corresponding to observable differences in the number of Azotobacter, led to an investigation of the cause of the apparent discrepancy. The three experiments just described are taken from and illustrate typical results obtained in this study. The data presented indicate rather conclusively that the *rate* of nitrogen fixation on washed agar plates is proportional to, or is a function of, the number of colonies, whereas the *quantity* fixed (with ample incubation period) is independent of either the number of organisms or the number of colonies. The quantity of nitrogen fixed is probably primarily dependent upon the supply of available energy. Barring possible variations in the efficiency of different species or strains of Azotobacter and other minor influencing factors, the quantity of nitrogen fixed in any culture is, therefore, independent of the number of organisms introduced into the culture provided ample time is allowed for the complete utilization of the food by the smaller number of organisms. The foregoing being true, one should not expect to be able to detect differences in the nitrogen fixing ability of different soils if the incubation period employed is relatively long, as has been the case in many investigations. On the other hand, if the incubation period is relatively short those cultures containing the larger number of Azotobacter may be expected to show a more rapid utilization of food and hence a more rapid fixation of nitrogen.

In the case of mixed soil cultures the total quantity of nitrogen fixed may be influenced through the utilization of the food by other organisms, particularly if fixation is slow and the period of incubation long. As soon as a limited quantity of nitrogen has been fixed by Azotobacter some of the nitrogen may become available to heterotrophic organisms thereby making conditions suitable for their growth with the consequent assimulation of food that otherwise would be utilized by Azotobacter. It is a common observance that the growth of molds, for example, is very abundant in such cultures after 7 to 14 days incubation. Contaminating organisms may also actually cause a loss of nitrogen through the transformation of some of the fixed nitrogen into a volatile form such as ammonia. The lower fixations recorded in tables 1 and

2 for the 21-day period with the fewer numbers of colonies may have resulted from one of these conditions.

The important point to be emphasized through these data is that differences in the nitrogen fixing ability of different soils, corresponding with differences in the plate counts of Azotobacter, can be detected provided a suitable period of incubation is employed. These data do not definitely show just how long this period should be for best results. It is quite evident, however, that the customary 3-, 2-, or even 1-week period is too long. On the other hand, sufficient time must be given so that the quantities of nitrogen fixed can be easily measured quantitatively.

Furthermore, the rate of growth and therefore of nitrogen fixation may vary markedly on different media and under different conditions, hence it is questionable whether one can select experimentally an incubation period that would be universally desirable. Of late it has been the policy in this laboratory to examine the cultures daily and make quantitative nitrogen analyses as soon as the appearance of the cultures indicates rapid fixation of nitrogen; the same incubation period being employed for all cultures to be directly compared. This procedure has resulted in detecting marked differences in the nitrogen fixing abilities of soils which by former methods exhibited no differences in this respect.

Experimentation has been confined largely to nitrogen fixation by Azotobacter, but there is every reason to believe that the fundamental principles involved are equally applicable to other phenomena.

SUMMARY

Marked differences can be detected in the number of Azotobacter in different soils. Soils exhibiting widely different numbers of Azotobacter may show no differences in their nitrogen fixing abilities when measured by the usual methods with the customary 1-, 2-, or 3-week incubation period. If the incubation period is only 3 to 5 days, differences in nitrogen fixing abilities, corresponding to differences in the number of Azotobacter, may be detected by the use of the plate method.

THE ZONAL SEQUENCE OF SOIL PROFILES IN SASKATCHEWAN, CANADA[1]

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The primary aims of this paper are to portray the general arrangement of soil profiles in the central or Saskatchewan portion of the semiarid and subhumid region of western Canada; to discuss their comparative extent and morphology; and to correlate briefly the more important profiles with the fundamental factors of their development.

As indicated in the title, the profiles will be considered as a sequence of zonal groups, extending from the short grass plains bordering the Montana-Saskatchewan segment of the international border, into the zone of woods and muskeg in the far north. Profiles will be described chiefly in terms of morphological characters, as it is planned to present chemical profile data in a later paper.

The writer feels that for at least two reasons the region under discussion may merit a claim to somewhat more than usual interest to soil scientists of this continent. In the first place there is no other region in North America where soil conditions so nearly resemble those described for Russia by that school of soil scientists which has done so much for the promotion of the genetical viewpoint of soil classification. A number of Russian soil scientists who have visited the region have expressed the same belief. In fact, so nearly does the general profile sequence of the region correspond to that of Russia in morphological character that Russian terms are used freely in soil descriptions. This striking relation is not surprising when one considers the type of existing natural environment: a northern, continental climate with a range of zones including semiarid and subhumid grasslands, park,[3] forest, forest-tundra, and possibly tundra. The other claim to somewhat more than usual interest may be made on the basis of the rather unique position occupied by the region relative to the great soil and environment zones running north and south through the heart of the continent. It is in the prairie provinces[4] of Canada that we find the great plains and prairies terminating in what might be termed the "Canadian grassland loop." Here we find the short grass plains terminating with an elliptical boundary and the taller grass belts (park and park-plains zones of Saskatchewan) turning westward to turn again southward along the east side of the Rockies. In other words, a point in the region of influence of cold northern temperatures has been reached where moisture efficiency increases from south to north moreso than from west to east without an appreciable increase in precipitation. Along the Rocky Mountain range the change is from east to west, a result of the influence of that mountain chain on climate.

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[1] The information upon which this paper is based has been accumulated in connection with the Saskatchewan Reconnaissance Soil Survey of the settled part of the province, now nearing completion. This paper is in the general nature of a preliminary regional report of the soils.

[2] Professor of soils, University of Saskatchewan, Saskatoon, Canada.

[3] "Park" is the term applied to a region with a native vegetation of grasses with scattered clumps of poplars and willows. These clumps usually occur surrounding small poorly drained areas.

[4] Alberta, Saskatchewan, and Manitoba.

These changes in climatic environment are accompanied by parallel changes in native vegetation and soils. Discussions of the environment zones with accompanying sketch maps have already been given by Joel (2, 3, 4) and by Wyatt and Newton (1, 5). The first attempt at a zonal soil map of the prairie provinces (unpublished) was presented at the Washington Soils Congress by Wyatt, Joel, and Ellis of the provinces of Alberta, Saskatchewan, and Manitoba, respectively.

THE ZONAL GROUPS OF SOILS OF SASKATCHEWAN

These groups represent the highest recognized categories in the present genetical classification of the soils of Saskatchewan. They reflect soil differences associated primarily with variations in climate and native vegetation in their broader aspects. These zonal and zonal transition groups correspond in categorical value to the zones already established in that they are broad belts of soil corresponding in general to climatic zones.

Zonal and zonal transition groups of soils arranged as a sequence and correlated with climate and native vegetation

1. Soils of the short grass plains, brown soil zone.
2. Soils of the dark brown (chestnut) soil zone.

3a. Soils of the shallow dark portion of the park zone (northern park).
3b. Soils of the deeper dark portion of the park zone (eastern park).

4. Soils transitional to the park and forest soil zones.
5. Soils of the forest gray soil zone.
6. Soils transitional to the forest and tundra soil zones.

These groups are arranged in progressive order from the warmer, drier grasslands to the colder, more moist forest and forest-tundra. At the same time the classification (except for group 6) is made on a basis of definite soil character. The basis used is what might be termed a "zonal-parent material association of soil profiles." In other words, each zonal group or zonal transition group is defined by an association of normal soil profiles typical of all the important parent materials occurring within the zone or zonal transition. Differentiation of the groups is based on variations in the normal profiles on like parent materials, such variations being easily recognized as resulting from differences in the influence of climate and native vegetation. The term "normal profile" in this case refers to the profiles typical of positions of average drainage and smooth topography, and of sufficient age to produce, to a reasonable degree, the characteristics which will be present in the mature profile.

The consideration of profiles on all important parent materials as a criterion for zonal group differentiation is contrary to some claims made for the dominance of climate over parent materials as a soil development factor. For Saskatchewan at least the writer is convinced that in some cases parent materials are fully as important as climate and native vegetation in determining soil character, and that in the majority of the soil types of the province parent materials leave a very distinct stamp on the profiles. This point will be further discussed in the latter part of the paper.

Although six soil-environment zones and zonal transition groups have been listed in the classification for Saskatchewan, only the first five have been studied by soil surveys or exploration. As far as the author is aware no soil scientists have yet studied the forest-tundra zone in Saskatchewan.

All normal soils of at least the first five groups that have been studied belong to the pedocal group (Marbut). Even in the wooded podzolic soils of the north a definite zone of lime concentration occurs, usually within 2 to 3 feet of the surface.

In the grassland groups the zonal influences are reflected by a progression in darkening of color of surface soil, in the accumulation of organic matter, and in the depth to the horizon of lime concentration, in the order in which the groups are given.

In the wooded soils there is, of course, a very great change in profile character as compared to grassland types.

In the park group the dark grassland soil types are almost always far more extensive than the podzolic types. The latter are found under the scattered clumps of trees characteristic of this zone.

Characteristic variations in profile within the zonal groups are brought out in the following classification showing the important zonal subgroups.

THE ZONAL SUBGROUPS OF SOILS OF SASKATCHEWAN

In the following classification are given the more important subgroups found within each of the first five zonal and zonal transition groups. These secondary categories are not established on a purely genetical basis. It is a grouping intended primarily to show the dominant types of profiles of varying morphological character, and to indicate their comparative importance from the standpoint of area occupied. Most of the descriptive names indicate the recognized genetical soil groups of the world to which the soil belongs. Where other terms are used the soils have not yet been classified as recognized genetical types. The silty clay soils have been listed in a separate group because of the striking contrast of their profiles with those of recognized genetical types.

The subgroups are listed in order of the author's estimate of their importance as to comparative area covered.

Zonal subgroups arranged to show their comparative extent within the zones and zonal transitions

1. Soils of the short grass plains, brown soil zone.
   - A. Soils covering extensive areas.
     - I. Brown solonetz and solodi (frequently occurring as a complex).
     - II. Solonetz-like soils.
     - III. Silty clay group (Sceptre Series).
   - B. Soils of frequent occurrence but local in distribution.
     - I. Salines.
2. Soils of the dark brown (chestnut) soil zone.
   - A. Soils covering extensive areas.
     - I. Chestnut or dark brown solonetz and solodi (frequently occurring as a complex)
     - II. Silty clay group (Regina Series).
   - B. Soils of frequent occurrence, but local in distribution.
     - I. Salines.
     - II. "Bluff"* podzolic soils (near the border of the park zone).

3a. Soils of the shallow dark portion of the park zone (northern park).
   - A. Soils covering extensive areas.
     - I. Shallow dark chernozems.
     - II. Shallow dark solonetz.
     - III. Silty clay group (Elstow Series).
   - B. Soils of frequent occurrence but local in distribution.
     - I. "Bluff" podzolic soils.
     - II. Salines.
     - III. Shallow dark solodi.

3b. Soils of the deeper dark portion of the park zone (eastern park).
   - A. Soils covering extensive areas.
     - I. Deeper dark chernozems.
     - II. Rendzina-like soils.
     - III. Deeper dark solonetz-like chernozems.
   - B. Soils of frequent occurrence but local in distribution.
     - I. Degraded chernozems and rendzina-like soils (of "bluffs").
     - II. "Bluff" podzolic soils.
     - III. Salines.

4. Soils transitional to the park and forest soil zones.
   - A. Soils covering extensive areas.
     - I. Dark gray degraded chernozems, shallow phase.
     - II. Dark gray degraded chernozems, deeper phase.
     - III. Dark gray degraded rendzina-like soils.
   - B. Soils of frequent occurrence but local in character.
     - I. Saline peats.
     - II. Acid peats.
     - III. Deep and shallow dark chernozems and rendzina-like soils.
5. Soils of the forest gray soil zone.
   - A. Soils covering extensive areas.
     - I. Upland podzolic soils.
     - II. Acid peats.
     - III. Saline peats.
6. Soils transitional to the forest and tundra soil zones.

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* "Bluff" is the term most commonly used in western Canada for the clumps of poplars and willows scattered over the park zone and to some extent over the dark brown soil zone.

COMPARATIVE MORPHOLOGY OF PROFILES

As indicated in the subgroup classification it is evident that a number of analogous groups of soils have been recognized as occurring in different zones and zonal transitions. This is of course the result of similar factors of soil development (parent materials, high water table, effect of salts, etc.) occurring in the different environment zones. To minimize the number of profile descriptions and to emphasize the dominant morphological profile types only a limited number of profiles have been selected for detailed descriptions. These may be considered as a key group of profiles in which most of the important morphological characters occurring in Saskatchewan soils are represented. Each profile is selected as being the best representative of its particular morphological type, the closely related types usually being mentioned following the profile description.

Key group of profiles representing morphological types

1. Brown solodi.
2. Salines of brown soil zone.
3. Chestnut solonetz.
4. Silty clay (Sceptre and Regina Series).
5. Shallow dark chernozems.
6. Deeper dark chernozems.
7. Rendzina-like soils.
8. Dark degraded deeper chernozems.
9. "Bluff" podzolic soils.
10. Upland podzolic soils.
11. Saline peat.

Description of key profiles

1. Brown solodi. (*a*) Grayish brown powdery mulch, usually less than 1 inch deep. (*b*) Thinly laminated, soft lumpy, brown horizon, usually noticeably silty and easily pulverized. (*c*) A horizon of faintly long cloddy or prismatic macro structure and laminated micro structure, grayish light brown in color, usually of the lightest texture of the profile and readily pulverized. (*d*) A horizon of round topped columnar structure with grayish top coatings, nutty to fragmental micro structure, dark brown or dark grayish brown in color, high in colloids, very sticky when wet, very hard and with marked shrinkage cracks when dry. A characteristic feature in connection with this horizon is the peculiar bleached and flattened roots of native grasses passing through the cracks between the fragments. (*e*) A horizon of high concentration of lime and frequently of alkali salts, particularly gypsum and sodium sulfate, structureless to faintly long cloddy, of about medium consistency. (*f*) A dark gray horizon with splotches of lime and salts, usually faintly thick platy in structure.

Depths have not been given for the aforementioned horizons, as they vary greatly, not only for different texture and parent materials but also with hardly noticeable differences in surface elevation. As a consequence a broad cut in the natural soil reveals a very uneven profile with the tough B_1 horizon (above the salts) in the form of an uneven wave and the grayish brown A horizons extending downward as tongues.

The structural profile described is widespread in the grassland and park zones. In fact, well-developed solodi profiles may be found within a few yards of podzolic types along the fringe of the forest. It is the dominant profile of the semiarid brown soil zone. Variations in color and organic matter content occur in the various zones. Closely related morphological types are dark brown, shallow dark, and deeper dark solodi.

This soil is particularly interesting in that it gives rise to the so-called "burnt-out" or clay spot soils of Saskatchewan and the "scab" lands of northern Montana. Apparently in these lands the softer, lighter textured A horizons have been removed in spots, probably by wind, exposing the top of the B_1 heavy horizon as patches of depressed, heavy, hard surface soil.

2. Salines of the brown soil zone. The profile discussed under this heading refers to the true salines, that is, those with salts throughout the profile and usually with an efflorescence of salts on the surface in dry weather and also without solonetz development noticeably progressing. The profiles of this group have not been as intensively studied as those of extensive zonal types. It seems certain, however, that there is seldom a well-defined structural profile. There seems to be no defined macro structure, but a strong tendency toward a granular micro structure, particularly in the upper part of the profile. As to color there seems to be a general arrangement of a dark gray portion over a light gray or bluish gray, over a mottled brown and bluish gray.

The salines are of local occurrence, but widespread over the grassland and park zones, occurring even at the fringe of the forest. They seem to be free of solonetz development only in places permanently moist. The thickness of the humus-bearing horizons vary according to zones, but not as definitely or as sharply as in upland well-drained soils. In the park-forest zonal transition and in the fringe of the forest zone many of the peats are saline to the surface.

3. Chestnut solonetz. (*a*) A grayish brown powdery mulch, usually less than one-half inch thick. (*b*) A dark or chestnut-brown horizon with a suggestion of thin foliation or lamination in the upper part of the horizon and prismatic or long cloddy structure below. (*c*) A heavier, darker brown, more compact horizon of prismatic or long cloddy structure, the clods of which readily break horizontally into flat-topped fragments or thick plates. Frequently there is a sharp line of cleavage between this and the humus containing horizon above, resulting in characteristic flat-topped prisms of various shapes. The lower part of the long cloddy horizon is usually yellowish gray in color. (*d*) A brownish or yellowish gray or dark gray horizon of lime concentration, the lime frequently occurring in small masses or concretions. This horizon is easily penetrated and pulverized, frequently resembling a low grade marl in consistency and appearance. (*e*) A dark gray to bluish gray upper C horizon usually containing fine flecks of limonite and occasionally faint brown stains or streaks. The structure of this horizon is usually faintly platy.

Soils of the structural profile described are widespread in the brown and dark brown zones, and in the shallow dark portion of the park zone. Colors, depth to the horizon of lime concentration, size of the long clod aggregates, and other factors vary considerably, especially in relation to zones and parent materials. In the heavier textured soils the clods are distinctly narrower, and the whole developed profile is shallower and less distinctly developed than soils of lighter textures. The color of surface horizons darkens and the depth to the horizon of lime concentration increases from the brown to the park soil zones.

4. Regina and Sceptre silty clay group. A close examination usually reveals a solonetz development to a very shallow depth. In soils mapped as light clays the development usually extends to a depth of 4 to 6 inches. In the very heavy clays it is frequently confined to a depth of not more than an inch and a half. Below this the structure changes gradually from small fragmental or cloddy to large clods 1 to 2 inches in diameter, several feet below the surface. The color ranges from dark gray tinged with brown near the surface to gray or moderately dark gray below. Small splotches of lime carbonate occasionally show faintly below the first foot from the surface.

In these soils horizons are not distinct. The soils take on a fine granular surface structure when cultivated for a few years, and consequently drift badly. They are, for this reason frequently known locally as "loose tops." They have a high content of silt and clay and therefore shrink greatly when dry, forming large cracks in the natural sod and large clods in the lower profile.

5. Shallow dark chernozem. (*a*) A very dark brown, almost black horizon, usually not more than 6 inches deep, of soft cloddy structure, which breaks readily into fine aggregates, mostly of granular structure. (*b*) A very dark brown somewhat thicker horizon, slightly heavier and soft cloddy, faintly prismatic in structure. (*c*) A coffee-brown or light rusty colored horizon, distinctly heavier than the soil above, and frequently faintly long cloddy in structure. (*d*) A gray, brownish gray, or dark gray horizon of lime concentration, and a dark gray upper C horizon resembling similar horizons described for profile 3.

This is the dominant profile of the shallow dark portion of the park zone. It seems to be closely related to the solonetz-like soils.

6. Deeper dark chernozem. In this profile there is distinctly more of granulation and but little suggestion of prismatic structure as compared to the usual shallow dark chernozem. The whole profile is deeper than in the latter, the humus horizon usually having two to three times the depth of the analogous horizon in the shallow type. The depth to the lime horizon is greater and the concentration of lime far less marked.

7. Rendzina-like soils (of the deeper dark park). The profile of this group seems to be closely related to the deeper chernozems and the park zone salines. Moderately poor drainage and highly calcareous parent material seem to be the important factors responsible for their occurrence. They are comparatively inextensive in Saskatchewan.

As a rule there seems to be no definite structural profile. There is a strong suggestion of a fine granular structure in the upper 1 or 2 feet and no definite structure below. The most characteristic features of the profiles are a grayish black, highly calcareous surface, corresponding in depth to the humus horizons of the deeper chernozems; a horizon of light gray marly soil, faintly mottled with brown; and a darker gray lower soil frequently quite strongly mottled with brown.

8. Dark degraded deeper chernozem. These soils are morphologically about what one would expect of a chernozem approaching a podzolic type through degradation as the result of a timber cover. The profiles vary greatly depending upon the degree of degradation, the slightly degraded ones closely resembling the chernozems and those in advanced stages of degradation closely resembling the podzolic types. During the degradation process the dark color changes to grayish black, mottled with brown flecks, and later distinctly bleaches out, the structure in the upper profile changes from fine granular to foliated or platy and of the lower profile from prismatic or coarse granular to hard fragmental. At the same time the depth to the lime horizon is gradually lowered.

These soils are typical of the park-forest zonal transition which is comparatively inextensive as compared to most of the other zones or zonal transitions.

9. "Bluff" podzolic soils. (*a*) Coarse organic debris, mostly decaying leaves and twigs, very dark brown to almost black, usually 1 to 2 inches thick. (*b*) A light ashy gray horizon, thinly foliated above and thin platy below, easily pulverized, and usually very fine sandy in texture, 2 to 8 inches deep. (*c*) A dark, bluish to grayish brown, nutty to coarse granular, moderately heavy, compact horizon with some brown staining, especially along root channels. This horizon is about as thick as the gray horizon above. (*d*) A similar horizon with much more brown staining. (5) A structureless horizon distinctly mottled with bluish gray and brown.

The profile described has not been as thoroughly studied as most of the others described. It occurs under a cover of populars and willows growing around moderately poorly drained places in the park and chestnut-brown zones. The features of the upper part of the profile seems to be typically podzolic whereas those of the lower part are much like the poorly drained bog, or non-salty slough types. The profile approaches the upland poplar podzolic profile in character in the outer, higher part of the tree rings (called "bluffs") which surround the bogs of marsh grasses and sedges in the central parts of the sloughs.

10. Upland podzolic soils. (*a*) Organic debris, mostly decaying leaves and twigs, very dark brown to black, usually 1 to 3 inches deep, with a slight amount of coarse mineral soil in the lower part. (*b*) A thin, very dark brown horizon of finely divided organic and mineral matter. (*c*) An ashy gray, thinly foliated, easily pulverized horizon showing somewhat thicker folia at the base of the horizon. (*d*) A dark grayish brown, heavy, compact, ortsteinlike horizon, platy to coarse granular above and nut-like in structure below. (*e*) A lighter

grayish brown similar horizon of small nut-like structure. Faint bluish gray splotches frequently are also found in the two heavy horizons just described. (*f*) A gray, or brownish gray, lighter textured, far less compact horizon of lime carbonate accumulation, soft lumpy in structure or structureless. The depth to this horizon varies greatly with soil texture, parent materials, and local topographic position. It usually begins at about 20 to 30 inches from the surface. (*g*) A darker gray horizon, also highly calcareous, with a faint platy structure and reddish brown spots and concretions.

The foregoing description applies to the well-developed, upland, podzolic profile found under a poplar vegetation over much of northern Saskatchewan, in association with the lowland peaty profiles in the numerous muskegs or northern bogs. The profile seems to be about what one would expect with a timber cover in a region of limited rainfall (probably about 14 to 17 inches a year over most of the wooded zone). A matter of interest in connection with the profile is its close resemblance morphologically to the solodi profiles of the semiarid short grass plains zone. A striking difference is the absence of round-topped columns in the podzolic type.

11. Saline peat. The peat profiles have been studied comparatively little in Saskatchewan. Both acid and saline types occur. In the farther north acid peats are common under sphagnum moss, spruce, and associated plants. In the forest fringe and park zone, rushes, sedges, marsh grasses, and associated plants are more typical as the natural vegetation. The following description is of the profile occurring in what is probably the most extensive saline peat area in the park zone, the Waterhen Marsh.

(*a*) About 2 inches of very dark brown semi-decomposed peaty muck with bits of shells and occasionally a thin salt incrustation. (*b*) About 10 inches of dark brown, coarser, less decomposed peat, with numerous small roots and some fragments of shells. (*c*) A very dark brown, mucky peat, high in salts and with numerous fragments of shells. (*d*) A dark, brownish gray muck high in salts, and with numerous fragments of shells. (*e*) A bluish gray to dark bluish gray, heavy silty clay, with little organic matter and with very few fragments of shells except in the upper part of the horizon and with scattered brown staining, especially along root channels.

RELATIVE EXTENT OF VARIOUS SUBGROUP TYPES OF SOIL

The province of Saskatchewan extends about 700 miles northward from the international boundary and has an average width of about 360 miles, making a total area of approximately 250,000 square miles. Most of the northern half of the province has very little settlement, and much of it has not even been well explored. The well-developed and well-settled portion is confined to about the southern two-fifths of the province.

Of the province as a whole, about 60 per cent is occupied by the forest zone, about 14 per cent by the short grass plains zone, about 11 per cent by the shallow dark portion of the park zone, about 11 per cent by the dark brown zone, and the remaining few per cent by the deeper dark portion of the park, the park-forest, and the tundra zones. The figures are based on soil survey information for the settled part of the provinces and estimates of areas of forest cover for the northern part of the province. The figures are given merely as close approximations to be used in making comparisons of the various subgroups as to area occupied. Based upon these figures and the author's knowledge of the relative extent of the various subgroup types, the following statement may be given with justification.

Undoubtedly the podzolic profiles occupy the major portion of the province, and the peaty, muskeg types a large proportion. Most of these soils, however, lie in a region the larger part of which is considered unsuitable for ordinary northern agriculture such as is now practised in most of the settled and developed areas of western Canada.

Considering only the settled portion of the province, which extends on an average about 40 miles into the forest and forest-park zonal transition, the following are probably the dominant subgroup types: brown solodi and solonetz, chestnut solonetz, shallow dark chernozems and the silty clay types. The other extensive types, with their relative importance within each zone, together with the frequently occurring soils of relatively small area are indicated in the zonal subgroup classification.

SOME CORRELATIONS OF MORPHOLOGICAL CHARACTERS OF PROFILES WITH SOIL DEVELOPMENT FACTORS

It has already been stated in the first part of the paper that a number of great soil and environment zones running north and south through the central part of the continent either terminate or turn sharply northwestward and finally almost westward in Saskatchewan. A point has been reached where the plains-to-forest sequence of soils takes place from south to north rather than from west to east.

A consideration of climative conditions would seem to indicate strongly that the aforementioned change in direction of zonal sequence of soils is largely the result of a change in temperature and precipitation relations. In the part of Saskatchewan in which the zones run only gently northwestward there is a definite increase in precipitation eastward across the zones, as in the United States. However, in the part of the province where the zones have turned almost westward there is apparently no increase in total or seasonal precipitation, but a decided decrease in mean annual and mean seasonal temperatures northward across the zones. It is also commonly believed that there is less total wind. There are no worthwhile records to substantiate this claim as to air movement, but the probabilities are that going northward there is at least a decrease in the dry, warm Chinook type of wind so common in the semiarid part of the province.

It seems justifiable to state therefore that the westward turn of the soil-environment zones in Saskatchewan is associated with an increase in moisture efficiency from south to north rather than from west to east; and that such increase seems to be associated with a decrease in mean temperature and warm, dry winds rather than with an increase in precipitation.

The effect of elevation on zonation of soils is well illustrated in a number of places in Saskatchewan. The best example is in the Cypress Hills. This preglacial plateau rises from 500 to 1,500 feet above the semiarid plains surrounding it. In a trip covering not more than a dozen miles one may pass from typical semiarid brown soils through park chernozems to podzolic types.

Practically all those who have done an appreciable amount of field work in connection with the Saskatchewan Soil Survey agree as to the marked influence of parent materials on the character of soil profiles, particularly in the grassland and park zones. In fact, in the case of the various silty clay groups of soils listed as subgroups in the classification given in the earlier part of the paper, the influence of the parent material on soil character is greater than that of the natural environment. These soils are derived chiefly from Pierre shales and lacustrine deposits. Soils from most other parent materials show distinctly the stamp of environment when they are compared in adjacent environment zones. The silty clay soils, however, show very little difference in profiles except in widely separated zones. Furthermore, the normal profiles of these soils are distinctly different from others within the same zone but developed on other parent materials. The extremely heavy texture of the silty clay soils is undoubtedly one reason for this.

In further reference to parent materials it is evident that the solodi profiles are far more prevalent on silty lacustrine deposits and deposits with a strong admixture of preglacial materials than on deposits of glacial till carried from the north. It is also evident that podzolic types are far more prevalent and better developed on well-drained glacial till than on silty lacustrine deposits.

This paper would not be complete without a brief discussion of an all important soil factor in Saskatchewan, namely, the importance of soil complexes. I is an extremely important consideration, not only to soil surveyors, but to experimentalists and farmers. By the term "complex" the author refers to distinct heterogeneity of profiles in a comparatively small area. The author has tiven particular attention to this matter in the course of field studies over much of the settled part of the province, and will likely later publish a paper covering this work. In this paper, however, it is intended to make only general observations.

The complexes are of at least two distinct groups, first, those including profiles of different zones and, second, those with profiles only of subgroups of the same zone. Both types of complex are usually associated with variations in local topography, parent materials, salt regime, or combinations of these.

Commonly occurring zonal complexes are podzolic, degraded, and chernozem or rendzina-like soils; "bluff" podzolic, shallow chernozem, chestnut solonetz, and chestnut solonetz-like soils. Commonly occurring zonal subgroup complexes are solonetz, solodi, solonetz-like soils and salines; and chernozem, solonetz, solonetz-like soils, and salines in various combinations.

Complexes resulting from distinctly different parent materials are fairly common, as with clays, loams, and sandy soils all in a very small area as the result of mixing of Pierre shales, bowlder clay, and sandy material by glacial action.

Probably the most prevalent of all types of soil complex in Saskatchewan is the one correlated with local topographic differences. Even very slight differences in local elevation, sometimes hardly perceptible, frequently result in marked variations in profiles within as small an area as a square rod of soil. This is especially true where solonetz and related genetic soil types are prevalent. Differences in erosion and deposition, in the amount of runoff water and of water percolating through the soil, in the influence of ground water, and in the kind and quantity of salts may occur in a variable topography. As a result there is not only distinct variation in soils from zone to zone but frequently great local complexity.

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PLATE 1

FIG. 1. A very heavy phase of silty clay (Sceptre Series) showing great shrinkage and cloddy structure with drying.

FIG. 2. A well-developed solodi showing soft cloddy A_1; thinly foliated, faintly prismatic A_2; heavy, columnar-fragmental B_1; horizon of lime concentration, B_2, with high salt concentration at base.

FIG. 3. Solonetz soil with soft cloddy A_1; faintly prismatic A_2 with suggetion of thin foliation; heavier prismatic or long cloddy B_1; and horizon of lime concentration, B_2.

FIG. 4. Gradation from shallow dark park profile of open grassy glade (right) to "bluff" podzolic profile on left.

FIG. 5. "Nest of eggs" or "keg-top" columns characteristic of top of B_1 horizon of solodi.

FIG. 6. Structural horizons of brown solonetz soils (described from left to right): Large prismatic, narrower prismatic, prismatic-lumpy, and horizon of lime concentration, total depth represented, about 22 inches.

FIG. 7. Part of a collection of over 90 monoliths in soil museum of Soils Department, University of Saskatchewan. Most soil types of the province are now represented in this collection. Left to right, saline with slight solonetz development, solodi, saline, solonetz, solonetz-like soil, and silty clay.

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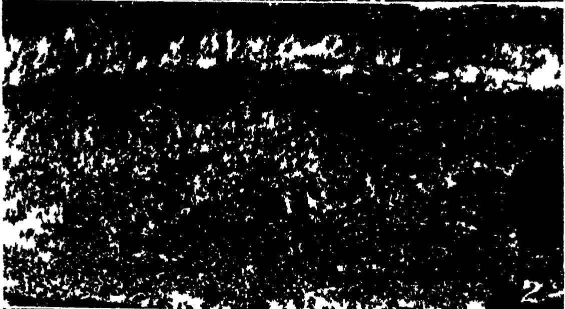
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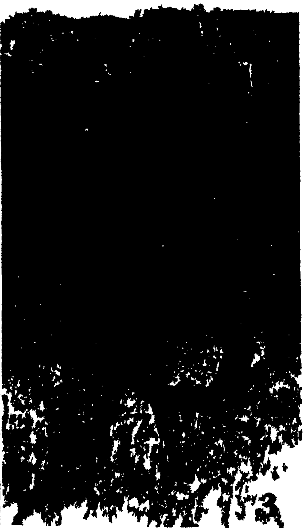
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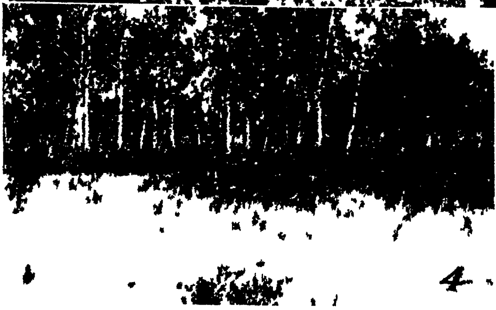
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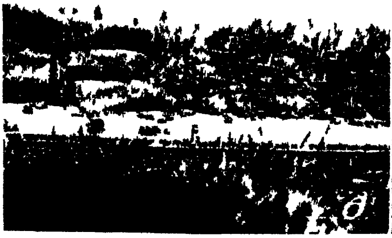
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PLATE 2

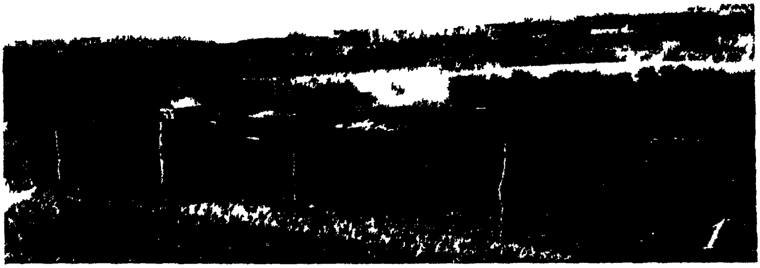
Fig. 1. Typical park zone country showing scattered "bluffs" (clumps of populars and willows) with grassland between. "Bluff" podzolic and shallow chernozem types predominate in such areas.

Fig. 2. Typical virgin land of semi-arid plains brown soil zone. Solonetz, solodi, solonetz-like soils, and Sceptre silty clays dominate in this zone.

Fig. 3. View over a northern wooded area in which podzolic and peaty muskeg soils are the principal types.

Fig. 4. A patch of "bluff" pozolic soil with shallow chernozem in background, in a field of new "breaking" along border of park and park-forest zonal transition.

Fig. 5. Complexity of soil in Saskatchewan is frequently associated with closely intermixed parent materials. Masses of Pierre Shale and bowlder clay have been thrown together by glacial action in this instance.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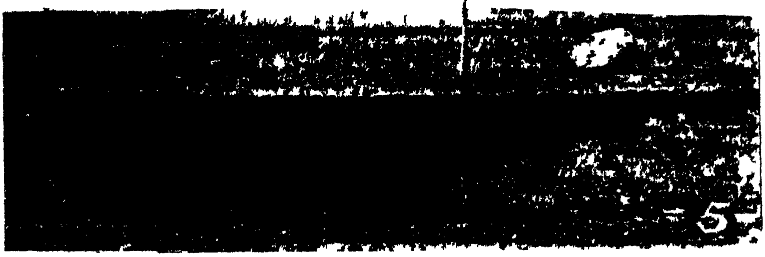
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ROOT DEVELOPMENT OF PERENNIAL GRASSES AND ITS RELATION TO SOIL CONDITIONS[1]

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It is generally recognized that roots play an important part in the growth of higher plants, since they serve as anchorage organs, repositories of food reserves, occasionally as organs of propagation, and regularly as absorbing organs. Practically all the water and minerals which the grass plant uses in growth are obtained from the soil solution through absorption by roots and root hairs. Obviously, the plant should be provided with a root system sufficiently deep and extensive to permit absorption of the moisture and nutrients required to support the top growth.

Since the roots of plants must actually occupy the various soil depths to obtain the moisture and nutrients contained, the ability of the root systems to invade and permeate the several depths is of extreme importance. The actual physical and chemical conditions present in the soil and the inherent response of the plant species under consideration to the stimuli at hand will determine root development and occupation of the soil layers.

The behavior of roots of crop plants has been the subject of considerable investigation during the past quarter century. The findings are reviewed by Weaver (11) and by Weaver and Bruner (12) in monographs on the root development of crops. The majority of the extensive field investigations of Weaver and his colleagues were conducted on soils classed as pedocals by Marbut (8), or on the prairie soils of the pedalfer group. Root behavior on such soils is not typical of that found on the podzolic soils of the eastern half of the United States, even though the species of plant remains the same. Further observations on the root habits of cultivated plants on the eastern agricultural soils are necessary in order that cultural practices may be so adapted as to fully utilize the resources available in soils of the region.

Cultural practices which modify the physiological condition of the plant, particularly the ratio of carbohydrate food reserves to available nitrogen, have been noted by many workers to have a profound effect on the development of both roots and tops. In addition, the residual effect on the soil of applying various fertilizers and soil amendments has come to be recognized as a potent factor influencing plant growth. Studies of root behavior of cultivated plants on podzolic soils, with various systems of culture, should eventually lead to a more rational and effective use of both soil and plant resources. The studies here reported are applicable, primarily, to the growing of grasses for pastures, lawns, and recreation fields on the gray-brown forest soils of the eastern states.

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[1] Journal series paper of the New Jersey Agricultural Experiment Station, department of agronomy.

The research here reported was supported in part by the New Jersey State Golf Association and the Metropolitan Golf Association.

[2] Acknowledgment is made to Dr. Nandor Porges for analyzing a portion of the soil samples, and to G. W. Burton for certain of the root harvests and soil analyses.

METHODS OF STUDY

After a period of experimentation concerned with methods of harvesting grass roots, the following procedure was adopted: Samples of turf were obtained from several representative areas by means of a special iron tool[3] having a straight blade 12 inches long, 7 inches wide, and ½ inch thick at the upper end, tapering gradually to the cutting edge. The tool blade is welded to an iron handle and has sufficient weight and durability to endure practically any treatment found necessary for insertion in the soil. A preliminary wedge-shaped opening was made in the sod to the full depth of the blade. A prism of soil was then sliced off approximately 1 inch thick, 7 inches wide, and 10 to 12 inches deep. This was carefully transferred to a wooden form and trimmed with a sharp knife to a width of exactly 4 inches and a thickness of ½ inch. The trimmed prism was then placed in an open box having a screen bottom with cross pieces studded with long brad points to hold the soil and roots in position. The soil was washed from the roots, after which the roots were divided into 1-inch lots according to the soil layer occupied while in place in the field. When thoroughly clean, the roots were dried and weighed.

The soil trimmed from the harvested prism was air-dried, screened, and stored for analysis of soil conditions associated with the root development. With root weights for each 1-inch layer and corresponding soil samples, it was considered profitable to attempt correlation of root occupation of the several layers with their specific soil properties. Determinations were made of the soil reaction by means of the potentiometer using the quinhydrone electrode. The readily available phosphorus was estimated according to Truog's (10) modification of Deniges colorimetric method. Organic carbon was determined by digestion with hydrogen peroxide and chromic acid as recommended by Degtjareff (2).

Because of the small size of the individual samples of sod harvested by this method, and the inevitable variations in soil characteristics even in contiguous areas, it is desirable that several samples be taken to represent each treatment or condition. Practically all of the data reported in this study are mean values of triplicate samples from typical areas.

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[3] This inexpensive tool was manufactured by the Council Tool Co., Wananish, North Carolina.

SEASONAL DEVELOPMENT OF ROOT SYSTEMS

The root systems of grasses in mature sod are completely adventitious in nature, and are periodically regenerated. The extent to which the root system is newly formed each year and the season or seasons when the development of new roots occurs, have not been known. Observations on these points were made in the spring of 1932 on 5-year-old sod of Kentucky bluegrass and Colonial bent grass maintained at a length of $\frac{7}{8}$ inch.

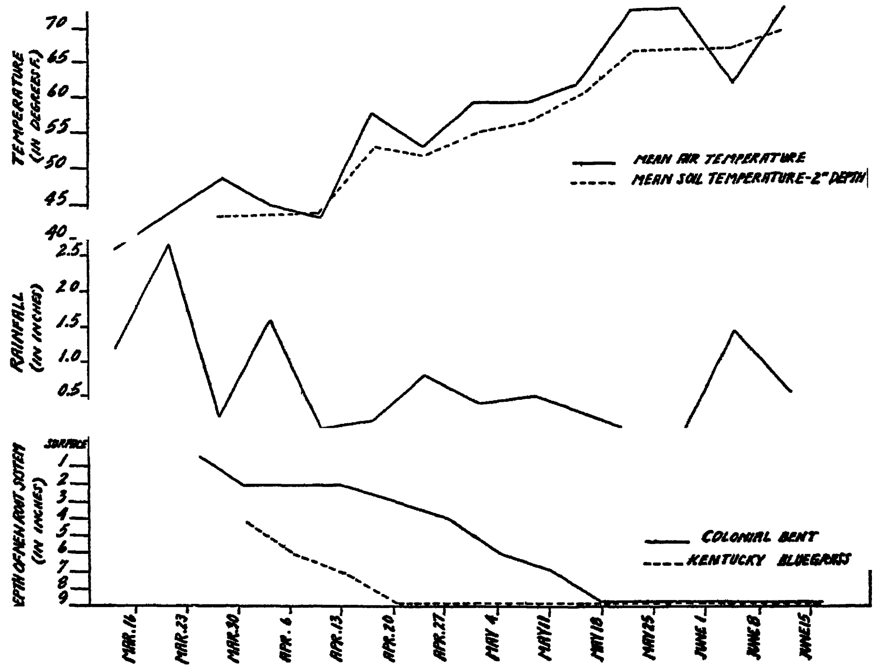

FIG. 1. RELATION OF TEMPERATURE AND RAINFALL IN SPRING TO THE EXTENSION OF NEW ROOTS BY KENTUCKY BLUEGRASS AND COLONIAL BENT GRASS

The soil on which the sod was produced is a normal member of the gray-brown forest soil group of the humid eastern states. The soil type is Sassafras loam, formerly cultivated, but in grass almost continuously for the past 9 years. The native fertility of this type is considered to be about average for soils of this region. Approximately 200 pounds per acre of a 4–16–4 fertilizer were applied annually from 1928 to 1930, inclusive. In 1931 and 1932 an 8–6–4 fertilizer was broadcast at a 400-pound rate in early spring.

The formation of new roots by these two species was closely correlated with temperature, as shown in figure 1. Kentucky bluegrass was found to be capable of root growth at lower temperatures than Colonial bent grass, a characteristic which is doubtless associated with the relative dates of blooming of the two species. New roots of bluegrass had reached a depth of 9 inches by April 20, whereas those of Colonial bent did not extend to that depth until May 18. Formation of new roots was practically completed by May 4 on bluegrass, but new roots continued to be formed intermittently on the bent grass until June 15.

The relative abundance of new and old roots is readily shown by comparing the total weight of roots throughout the soil layers occupied by the root system. The data on weight of roots of Kentucky bluegrass for successive dates are contained in table 1. The weights for the first inch have been corrected for the presence of underground stems; the actual weights of roots are given. It may be noted that the total root yield on April 6, when relatively few new roots had been formed, was approximately half as great as the maximum weight attained in early May. Root weight was reduced during the latter half of May when top growth was very heavy, but a gradual recovery occurred in June as the abundance of top growth became less.

TABLE 1

Weight of roots for Kentucky bluegrass at successive dates, in pounds under 1,000 square feet of surface, per inch layer

Date of root harvest, season of 1932

SOIL LAYER	APRIL 6	APRIL 13	APRIL 21	APRIL 27	MAY 4	MAY 11	MAY 18	MAY 25	JUNE 1	JUNE 8	JUNE 15	JUNE 22
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1st inch*	4.5	5.5	5.3	6.5	8.4	10.6	9.6	8.3	9.5	9.5	9.2	8.6
2nd inch	2.7	1.9	2.1	2.4	3.9	3.7	3.3	2.3	2.5	2.7	3.4	3.5
3rd inch	0.7	0.9	1.5	0.9	1.1	1.1	1.0	0.7	0.8	1.3	0.8	1.4
4th inch	0.6	0.6	0.7	0.7	0.8	0.6	0.6	0.4	0.5	0.8	0.5	0.7
5th inch	0.2	0.4	0.4	0.5	0.6	0.4	0.5	0.4	0.3	0.6	0.4	0.3
6th inch	0.1	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.2
7th inch	0.1	0.3	0.3	0.2	0.2	0.3	0.3	0.2	0.3	0.3	0.2	0.1
8th inch	0	0.2	0.2	0.1	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.1
9th inch	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0
Total........	8.9	10.3	10.9	11.7	15.6	17.5	15.9	12.9	14.5	15.9	15.1	14.9
Total below 1st inch....	4.4	4.8	5.6	5.2	7.2	6.9	6.3	4.6	5.0	6.4	5.9	6.3

* Weights in 1st inch are corrected for presence of stems.

It is significant that the abundance of roots below the first inch followed closely the trend of the total weight, including the upper inch. Since the separation of roots from stems is a tedious task, the expenditure of considerable time and effort may be avoided by basing conclusions on the weight of roots below the first inch where underground stems are usually not found. The high correlation noted between total root weight and root weight below the first inch, for both Kentucky bluegrass and Colonial bent, indicates that the roots in the upper inch may be eliminated from consideration without impairment of the validity of the data.

The behavior of Colonial bent roots was similar to that of bluegrass roots, the chief difference being that growth began later and the maximum weight was not attained until the latter part of May. From table 2 it is apparent that Colonial bent roots are distributed throughout the upper 9 inches in the same manner as those of Kentucky bluegrass. The roots of the two species were different in appearance; those of bluegrass were larger in diameter and darker in color than the roots of Colonial bent. These results are contrary to the popular opinion that Kentucky bluegrass has a denser but shallower root system than bent grass.

TABLE 2

Weight of roots for Colonial bent grass at successive dates, in pounds under 1,000 square feet of surface, per inch layer

Date of root harvest, season of 1932

SOIL LAYER	MARCH 30	APRIL 6	APRIL 13	APRIL 21	APRIL 27	MAY 4	MAY 11	MAY 18	MAY 25	JUNE 1	JUNE 8	JUNE 15	JUNE 22
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1st inch*	4.3	5.6	6.6	6.0	6.4	8.0	6.5	9.6	6.7	6.5	6.7	5.4	6.3
2nd inch	1.5	2.7	2.6	2.9	3.3	3.9	2.2	5.0	4.8	2.8	2.3	4.0	2.7
3rd inch	0.9	1.1	0.7	0.8	0.7	0.9	0.7	0.9	0.7	0.9	0.9	1.0	0.8
4th inch	0.4	0.5	0.4	0.3	0.3	0.6	0.4	0.5	0.3	0.9	0.7	0.4	0.6
5th inch	0.3	0.4	0.3	0.2	0.3	0.4	0.3	0.3	0.3	0.6	0.5	0.3	0.3
6th inch	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.1
7th inch	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.1	0
8th inch	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0
9th inch	0	0	0	0	0	0.1	0.1	0	0.1	0.1	0.1	0	0
Total...........	7.7	10.7	11.0	10.6	11.5	14.5	10.8	16.9	13.6	12.4	12.0	11.5	10.8
All roots below 1st inch.......	3.4	5.1	4.4	4.6	5.1	6.5	4.3	7.3	6.9	5.9	5.3	6.1	4.5

* Weights in 1st inch are corrected for presence of stems.

The response of the two species to seasonal temperatures is presented in figure 2. The data plotted were obtained by calculating from tables 1 and 2, moving averages of three consecutive harvests, to overcome the fluctuations due to sampling errors. It is obvious from the graphs of root weight below the first inch that bluegrass produced more roots in early spring and reached a maximum root weight earlier than Colonial bent. Aside from this time difference, the graphs for the two species are very similar, suggesting that both grasses pass through identical physiological cycles of growth, except that the response to temperature is initiated at a lower point for bluegrass than for Colonial bent.

It is of some consequence that fully half of the root system of these sod grasses is generated each spring. The kind and amount of nitrogenous fertilizer applied during this comparatively short period, together with the height and frequency of clipping or grazing, will obviously affect the occupation of the soil by the roots for the entire growing season. Practices that permit more extensive root development will allow the plant to draw on larger volumes of soil for nutrients and moisture, and consequently produce greater yields, or resist more unfavorable weather conditions than would otherwise be possible. The critical point is to determine with some exactness the effect of various treatments upon root development or abundance, on soils with known characteristics.

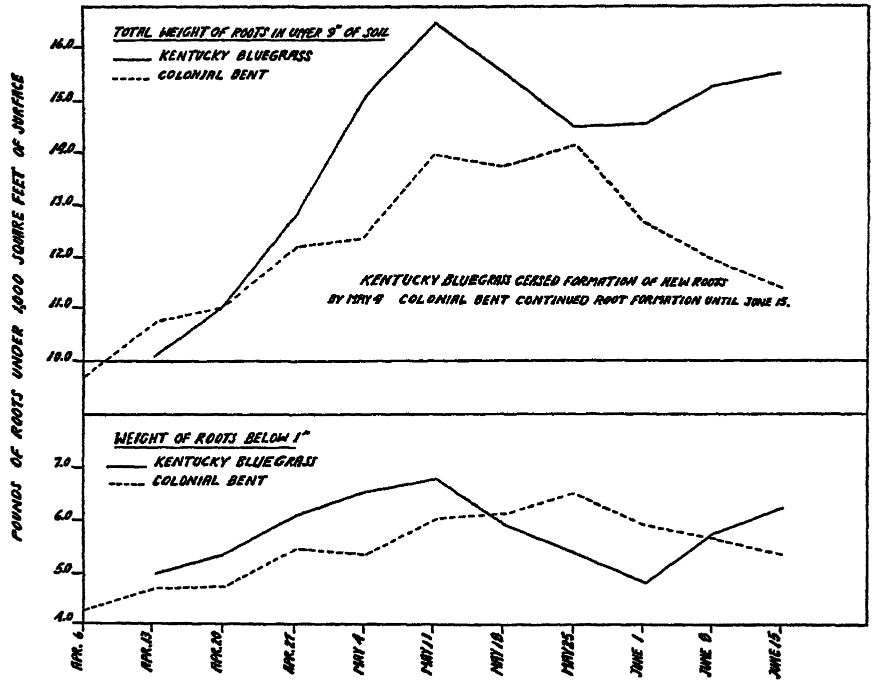

FIG. 2. PROGRESSIVE CHANGES IN DRY WEIGHT OF ROOTS OF KENTUCKY BLUEGRASS AND COLONIAL BENT GRASS UNDER 1,000 SQUARE FEET OF SURFACE

COMPARISON OF ROOT DEVELOPMENT ON DIFFERENT GRASS SPECIES

A detailed study of the root development of six species of grass was conducted in 1931 from roots harvested June 20 to 26. The grasses were all planted in the autumn of 1927 on Sassafras loam soil in the same field as that used in the foregoing harvests. An 8–6–4 fertilizer was broadcast in April 1931, at the rate of 400 pounds to the acre. In previous years, 200 to 400 pounds of a 4–16–4 fertilizer had been broadcast annually. The effect of occasional light applications of soluble nitrogenous fertilizers in 1928 and 1929 had disappeared by 1930. The soil type is well drained and the fertilization permitted vigorous growth of all the species examined.

In 1928, 1929, and 1930, one half-plot of each of six grasses was cut periodically at a length of ¾ inch to 1 inch. In 1931, these half-plots were mowed twice weekly at ⅞ inch. The corresponding half-plots of Colonial bent, velvet bent, seaside bent, and hard fescue were maintained at the ⅞ inch length until the spring of 1930, when the height of cut was lowered to ¼ inch. These closely cut half-plots were mowed twice weekly, which is less often than the common practice on golf course greens, and probably permitted more abundant root development than frequent mowing. The putting turf was top-dressed with a rich compost four times in 1930, and twice in 1931 prior to root harvest. In

TABLE 3

Comparison of root development on grass species, cut at different heights. Pounds of dry roots under 1,000 square feet of surface—New Brunswick, N. J., June 20–26, 1931

SOIL LAYER	COLONIAL BENT		VELVET BENT		SEASIDE BENT		HARD FESCUE		KENTUCKY BLUEGRASS		REDTOP	
	⅞ inch length	¼ inch length	⅞ inch length	¼ inch length	⅞ inch length	¼ inch length	⅞ inch length	¼ inch length	⅞ inch length	Uncut	⅞ inch length	Uncut
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1st inch*	71.2	70.6	68.9	92.1	87.8	67.0	81.6	94.5	125.2	129.7	92.3	125.5
2nd inch	13.1	12.4	15.1	13.8	20.4	8.9	12.4	11.4	18.5	18.2	11.0	13.0
3rd inch	6.6	3.8	7.3	4.5	7.0	4.2	9.9	10.6	9.6	7.9	6.1	7.5
4th inch	6.5	2.8	4.5	3.2	3.9	3.2	8.2	7.6	6.6	5.1	6.2	4.8
5th inch	3.8	2.0	3.5	3.1	3.1	3.5	5.9	4.9	5.2	6.2	3.2	3.8
6th inch	2.4	0.7	2.8	2.0	2.7	1.7	4.6	5.5	2.8	3.9	2.7	1.8
7th inch	1.4	0.6	1.8	1.8	2.1	1.1	8.9	3.9	3.1	3.0	1.7	2.3
8th inch	1.1	0.6	1.3	0.7	1.7	1.5	5.8	3.0	1.7	2.4	0.8	2.4
9th inch	0.7	0.3	0.7	0.3	1.1	0.3	3.0	3.2	1.5	2.2	0.7	0.4
Total root weight.....	106.8	93.8	105.9	121.5	129.8	91.4	140.3	144.6	174.2	178.6	124.7	161.5
Root weight below 1st inch.......	35.6	23.2	37.0	29.4	42.0	24.2	58.7	50.1	49.0	48.9	32.4	36.0
Average conditions in the upper 4 inches of soil												
Reaction of soil, pH..........	5.2	5.3	5.2	5.3	5.3	5.3	5.2	5.0	5.7	5.5	5.6	5.6
Available phosphorus, p.p.m..	152.0	170.0	205.0	192.0	127.0	125.0	78.0	82.0	131.0	141.0	138.0	152.0
Organic carbon, per cent......	2.4	2.3	2.5	2.0	2.2	2.1	2.0	2.0	2.0	2.1	2.1	2.4

* Weights in 1st inch are *not* corrected for presence of stems.

addition, a light fertilization with sulfate of ammonia was applied in May 1931. The corresponding half-plots of Kentucky bluegrass and redtop were cut once each year for hay from the time of their establishment.

The detailed results are presented in table 3. The weights recorded for the first inch include underground stems as well as true roots. As shown in the foregoing observations, the weight of roots below the first inch is a fairly accurate measure of total root abundance. Comparisons between species in this study are made on the basis of root weights in the layers containing no underground stems. There was little difference between the three bent grasses cut at ⅞ inch in abundance of roots, although the character of top growth was distinctly dissimilar. Hard fescue developed a far more extensive root system than the other species, and Kentucky bluegrass was definitely superior to red top. It is significant that when comparisons are restricted to the ⅞ -inch height of cut, the species having the greatest total root growth also displayed strong root development to the greatest depth. In spite of the considerable differences in habit of top growth for the six species, this relation of roots to soil applied to all. The abundant root development of hard fescue in the lower layers probably explains in part the ability of this species to survive under adverse conditions.

When the half-plots of the four species cut at ⅞-inch are compared with half-plots cut at ¼ inch, it is apparent that close mowing not only reduced the total root weight, but this reduction became more and more striking with increasing depth. The least change produced by close clipping was noted in hard fescue, although it is significant to observe that this half-plot almost completely died in early July, presumably because of the continued close mowing and depletion of food reserves.

Mowing regularly at ¼ inch length has been noted by Harrison (5) and others to induce scantier root development than cutting at greater lengths in species which tolerate both types of treatment. Close mowing reduces leaf area and the total quantity of food synthesized in the leaves, which in turn retards root growth. Furthermore, when a more liberal nitrogen supply is given closely cut grasses, top growth is stimulated at the expense of the carbohydrate reserves, and the development of the root system. Such behavior has been observed by Graber (3) and by Graber and Ream (4) for bluegrass, redtop, and fescue. The height of cut which permits maintenance of food reserves in the plant above the lethal level differs with the habit of growth. Thus, in these experiments the three bent grasses continued to make satisfactory growth with mowing at ¼-inch, whereas hard fescue was eventually killed by the same treatment. In the case of the bent grasses, close mowing induced a dense growth of basal leaves, probably providing for adequate synthesis of foods. Although close mowing of hard fescue induced tillering, the species was not able to increase the abundance of leaves below the level of cutting, sufficient to maintain adequate leaf area for food making. Continued depletion of food reserves by close mowing eventually caused starvation of the grass.

In the case of bluegrass and redtop, comparisons may be made between regular clipping at ⅞-inch length and mowing for hay once each year. Root growth of bluegrass was not restricted by regular mowing, indicating that this species is well adapted to maintenance of abundant leaf area near the soil surface. Redtop roots were less abundant where the tops were mowed regularly than when left uncut. This difference in behavior of the two species is correlated with their relative persistence in lawns. Kentucky bluegrass is a thrifty aggressive species on favorable soil types, whereas redtop tends to disappear from mixed turf in the course of 2 or 3 years, or to be replaced by other species when mowed regularly at a height of 1 inch or less. For bluegrass, prostrate growth of tops and the production of stolons were stimulated by frequent clipping. The behavior of bluegrass is apparently similar to that noted for Bahia grass by Leukel and Coleman (7), in which mowing scarcely affected the reserves of stored food in stolons and roots. Redtop, being a more upright species in habit of growth, was apparently reduced in total leaf area maintained and capacity for manufacture of food, and, consequently, root development was limited.

From the standpoint of soil conditions, the three bent grass plots were located in one portion of the field forming a natural group, whereas fescue, bluegrass, and redtop comprised a distinct group in a second area. The most significant differences in soil characteristics within the latter group are the lower pH and available phosphate values of the soil for the hard fescue plot. Since hard fescue possessed the most abundant root system of these grasses, it seems logical to conclude that these low values were due to the higher absorption of phosphorus and lime by the abundant roots of this species, rather than to the stimulation of root growth by low pH and phosphate values. The entire area occupied by the plots of fescue, bluegrass, and redtop had received similar treatment in previous years, and the soil should be similar in properties. The differences observed were doubtless caused largely by root occupation of the various grasses.

From the rate at which root abundance decreased with depth, one may conclude that practically all of the grass roots were to be found in the upper 12 inches of soil on areas cut at ⅞-inch length. The only exception noted was hard fescue. Mowing at ¼-inch reduced the working depth of those species capable of surviving under such treatment, to a working depth 2 to 3 inches less than mowing at ⅞-inch.

SOIL CONDITIONS LIMITING ROOT GROWTH

The determination of certain soil properties by 1-inch layers, and the corresponding abundance of roots, are shown for each of three grass species in table 4. No significant reason for the gradual reduction in root abundance with increasing depth was found in determinations of soil acidity, available phosphate, and organic carbon. Although pH values increased with depth, the lower readings in the upper layers doubtless were caused by greater absorption of basic materials by the roots.

Organic carbon values fell off sharply at the 9-inch depth for bluegrass and redtop, a condition also noted with the bent grasses. In the case of hard fescue, organic carbon content was maintained to the 9-inch level, indicating that the carbon supply was largely dependent on root occupation, rather than root occupation being conditioned by the presence of sufficient organic matter. Root abundance decreased at such a rate for bluegrass and redtop that the limit of penetration might be expected at about 10 to 12 inches. Since the reduction in the upper 8 inches was independent of the carbon content, there is no reason for assuming a causal relation at the 9-inch level.

TABLE 4

Relation of soil conditions at different levels, to development of roots by 3 species, all plots fertilized similarly and mowed twice weekly at ⅞ inch. Pounds of roots under 1,000 square feet of surface, harvested June 20–26, 1931*

SOIL LAYER	REDTOP				HARD FESQUE				KENTUCKY BLUEGRASS			
	Weight of roots	Soil reaction	Available phosphorus	Organic carbon	Weight of roots	Soil reaction	Available phosphorus	Organic carbon	Weight of roots	Soil reaction	Available phosphorus	Organic carbon
	lbs.	*pH*	*p.p.m.*	*per cent*	*lbs.*	*pH*	*p.p.m.*	*per cent*	*lbs.*	*pH*	*p.p.m.*	*per cent*
1st inch†	92.3	5.5	122	2.3	81.6	5.0	59	2.1	125.2	5.6	122	2.3
2nd inch	11.0	5.6	133	2.3	12.4	5.2	74	1.9	18.5	5.7	133	1.9
3rd inch	6.1	5.6	147	2.2	9.9	5.3	88	1.9	9.6	5.7	143	1.9
4th inch	6.2	5.7	152	1.9	8.2	5.3	84	1.9	6.6	5.7	125	1.9
5th inch	3.2	5.7	135	2.0	5.9	5.4	88	1.7	5.2	5.7	133	1.9
6th inch	2.7	5.7	136	1.9	4.6	5.4	82	2.1	2.8	5.7	137	2.0
7th inch	1.7	5.8	129	1.7	8.9	5.4	89	2.3	3.1	5.7	100	1.8
8th inch	0.8	5.8	86	1.5	5.8	5.4	69	1.8	1.7	5.7	45	1.2
9th inch	0.7	5.7	59	1.4	3.0	5.5	35	2.2	1.5	5.7	35	1.0
Total roots...	124.7	.....	.....	.....	140.3	.....	.....	.....	174.2	.....	.....	.....
Roots below 1st inch....	32.4	.....	.....	.....	58.7	.....	.....	.....	49.0	.....	.....	.....

* Fertilized in April 1931 with 400 pounds per acre of 8–6–4 fertilizer.

† Root weights in the 1st inch are *not* corrected for presence of underground stems.

The available phosphorus content appears to have been greatly reduced by absorption in the upper levels. The phosphorus content fell off sharply below the plow zone of 7 to 8 inches, and this was associated with a marked reduction in root abundance. In view of the uniform diminution in root development in the upper soil layers, independent of phosphate values, it seems likely that the low phosphate content of the soil layers at the 8- and 9-inch depths were not prime factors limiting further penetration.

The observations of Laird (6) on root systems of Bermuda and centipede grasses on light sandy soils and on such soils with a 3-inch layer of clay loam added to the surface, suggest that aeration of the respective soil layers may be an important factor influencing root penetration. Since the soil on which the observations here reported were made is a heavy loam that had not been plowed for 4 years, one might expect considerable differences in oxygen supply with increasing depth. No data concerning this factor were obtained.

Whatever the soil conditions may be that limit root penetration, hard fescue is apparently better adapted to utilization of soil layers having these inhibiting characteristics than the other species examined. One may speculate on the economic value of a species or strain which would combine the stoloniferous habit of growth of bluegrass with such vigorous development of the root system as was noted for the fescue.

RATIO OF ROOT GROWTH TO TOP GROWTH

The ability of a grass species to survive with repeated mowing is determined not to as great an extent by the total root growth, as by the ratio of roots to top growth which must be kept supplied with moisture and nutrients. Although a considerable amount of basal growth persists continuously below the height of cut which is not measured by harvest of clippings, nevertheless some relation might be expected between yield of cuttings and the withdrawal of moisture and nutrients from the soil.

The ratios of root weights below the first inch to the total dry weight of clippings from early spring to July 1 are given in table 5. The yield of clippings for this period was included since artificial watering was unnecessary in 1931 until the beginning of July, and comparisons may therefore be made directly between turf cut at different heights without the introduction of a moisture factor. It is obvious that each of the three bent grasses, and hard fescue, showed more favorable root-shoot ratios for maintenance of top growth, when cut at $\frac{7}{8}$-inch than when mowed at $\frac{1}{4}$-inch. This favorable relation combined with the greater volume of soil occupied by the longer turf satisfactorily explains the observed differences in susceptibility to injury from drought between the two heights of cut.

Comparisons between species cut at lawn length show a superiority for seaside bent and hard fescue. The favorable ratio for seaside bent was due primarily to low yield of clippings, whereas for hard fescue it was caused by a vigorous root development. The low ratio for Kentucky bluegrass was produced by heavy growth of tops, whereas in redtop a poor ratio was the result of scanty root development. From the standpoint of agricultural use, the vigorous root development of bluegrass and its high yield of cuttings, combined with the normal placement of creeping stems below the surface and the consequent protection from desiccation in unfavorable periods, give this species an advantage over any of the other species in this study.

For use as a turf grass on lawns and recreation fields, hard fescue is superior to bluegrass as regards the root-shoot relations. Yield of tops is of small consequence for such turf; in fact, a low yield is desirable, since less labor will be required to maintain the desired height of cut. Bluegrass is superior because of its stoloniferous habit of growth and ability to invade new areas or to heal injuries, which compensate for its slightly inferior root-top ratio. On very poor soils where survival is the all-important factor, hard fescue is undoubtedly superior, both from the standpoint of absolute root growth, and the ratio of roots to tops. Although the bristle-like form of the hard fescue leaves is suited to the reduction of water loss in dry periods, the root extent and root-top ratio probably account for a considerable portion of the drought resistant characteristic of the species.

TABLE 5

Weights of roots and clippings of various species of grasses, in pounds per 1,000 square feet of surface area—season of 1931

SPECIES OF GRASSES	GRASS CUT TWICE WEEKLY AT A LENGTH OF ¼ INCH			GRASS CUT TWICE WEEKLY AT A LENGTH OF ⅞ INCH		
	Weight* of roots from 1–9 inches only 6/23/31	Yield of clippings from April to July 1	Ratio of roots in 1–9 inch levels to total yield of clippings	Weight* of roots from 1–9 inches only 6/23/31	Yield of clippings from April to July 1	Ratio roots 1–9 inches to total clippings
	lbs.	*lbs.*		*lbs.*	*lbs.*	
R. I. bent	23.1	26.5	1:0.87	35.7	27.4	1:1.30
Velvet bent	29.5	26.9	1:1.10	37.1	25.3	1:1.47
Seaside bent	24.5	28.7	1:0.85	42.2	14.3	1:2.95
Hard fescue	50.0	66.7	1:0.75	58.7	27.2	1:2.16
	GRASS UNCUT AS FOR HAY			GRASS CUT TWICE WEEKLY AT A LENGTH OF ⅞ INCH		
Kentucky bluegrass	48.9	....	......	49.1	46.9	1:1.05
Redtop	36.0	....	......	32.4	29.5	1:1.10

* Weight of roots in the upper inch of soil was disregarded because separation of roots from underground stems was not made.

EFFECT OF FERTILIZERS ON ROOT DEVELOPMENT

Since the observations on root occupation and the characteristics of the several soil layers, presented in the foregoing, were not conclusive, further investigations were conducted with a single grass species on plots which had received distinctly different treatments. Root harvests were made on a series of plots receiving 12 types of fertilization, all occupied by the Virginia strains of creeping bent and mowed regularly three times a week at a height of approximately ¼-inch. The test plots were first fertilized in 1926 and have been subjected to the same treatment continuously since that time. The various treatments have produced greatly different types of growth, as reported elsewhere (9), in spite of the fact that equal amounts of nitrogen were supplied to all plots except those receiving manure or no fertilizer. The area included in these tests lies adjacent to that used for the studies discussed earlier and is comparatively uniform in character. Since several of the treatments were found to produce striking differences in soil reaction, and others provided nutrient elements in addition to nitrogen, some correlation between soil properties and root development was predicted.

TABLE 6

Weight of roots of Virginia creeping bent grass, at different soil levels, in pounds under 1,000 square feet of surface, as affected by various fertilizer treatments. Fertilized regularly since July 1926, roots harvested September 1-8, 1931*

SOIL LAYER	NO FERTILIZER	NITRATE OF SODA	SULFATE OF AMMONIA	AMMONIUM PHOSPHATE	5–10–5 FERTILIZER	ALFALFA MEAL	BONE MEAL	MANURE	NITRATE OF AMMONIA PLUS		NITRATE OF AMMONIA PLUS	
									Light sulfur	Heavy sulfur	Light lime	Heavy lime
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1st inch†	39.3	36.7	74.3	51.3	42.6	37.8	33.8	28.6	36.7	78.7	25.7	20.6
2nd inch	12.3	3.2	23.7	24.7	4.5	9.7	11.6	10.0	14.7	79.3	9.6	4.2
3rd inch	2.7	2.5	12.1	16.5	2.7	3.4	3.1	3.7	4.8	14.5	1.6	0.8
4th inch	2.0	1.3	3.8	3.1	0.8	1.7	2.1	0.8	2.7	6.6	0.8	1.3
5th inch	1.3	0.4	2.4	1.3	0.6	1.1	1.1	0.7	0.8	6.6	0.6	1.1
6th inch	0.7	0.4	1.4	1.0	0.4	0.6	0.7	0.6	0.7	4.8	0.3	0.4
7th inch	0.7	0.4	2.2	0.6	0.3	0.4	0.6	0.4	0.6	3.6	0.3	0.3
8th inch	0.4	0.4	1.3	0.7	0.3	0.6	0.4	0.3	0.4	1.7	0.4	0.3
9th inch	0.6	0.1	1.1	0.4	0.1	0.7	0.3	0.3	0.3	1.1	0.4	0.1
10th inch	0.3	0.1	0.3	0.3	0.1	0.3	0.3	0.1	0.1	0.4	0.3	0.1
Total root weight.....	60.3	45.5	122.6	99.9	52.4	56.3	54.0	45.5	61.8	197.3	40.0	29.2
Root weight below 1st inch.......	21.0	8.8	48.3	48.6	9.8	18.5	20.2	16.9	25.1	118.6	14.3	8.6
Average conditions in the upper 4 inches of soil												
Soil reaction, pH.	5.8	6.3	5.0	5.3	5.9	6.7	6.0	6.1	5.3	4.9	7.2	7.8
Available phosphorus, p.p.m.	47.0	59.0	39.0	89.0	227.0	58.0	392.0	68.0	69.0	44.0	146.0	93.0
Organic carbon, per cent......	2.1	2.2	2.4	2.3	2.2	2.3	2.2	2.4	2.4	2.2	2.2	2.0
Vigor of turf....	poor	medium	very poor	very poor	med. to good	med.	med.	poor	poor	poor	good	good

* All fertilizers were applied in four to five equal parts during the season, at rates to supply 2.83 pounds nitrogen per 1,000 square feet per year. Well-rotted manure applied at rate of 689 pounds per 1,000 square feet, in late winter.

† Root weights in first inch are *not* corrected for presence of underground stems.

The yields of roots by soil layers, and the average conditions in the upper 4 inches of soil are shown in table 6. Eliminating the weights for the uppermost inch because of the presence of creeping stems, the total root weights for the next 9 inches vary from 8.6 to 118.6 pounds under 1,000 square feet of surface. In general the treatments which had produced an acid reaction of the soil below a pH value of 5.5, were associated with high yields of roots. Thus, sulfate of ammonia fertilization had developed a reaction of pH 5.0 and permitted a root yield of 48.3 pounds, as compared with a pH of 6.3 and a root yield of 8.8 pounds for the plots receiving nitrate of soda. Similarly, the light sulfur and light lime treatments produced 25.1 and 14.3 pounds roots, respectively, although both were accompanied by identical applications of nitrate of ammonia.

The extraordinary yield of subterranean growth for the heavy sulfur treatment was caused by the presence of undecayed stolons in the second inch. The turf on all plots was top-dressed four times yearly with a screened mixture of two-thirds loam soil and one-third sand. In all plots with the exception of those receiving heavy additions of sulfur, the stolons were confined to the upper inch. It was characteristic of all acid plots, however, that a considerable portion of the roots appeared brown and non-functional. The conclusion may be drawn that many of the roots harvested on acid plots were produced in previous years, and still persisted because conditions were unfavorable for their decomposition. In the heavy-sulfur plots, stolons as well as roots failed to decay.

Irrespective of root abundance, the plots with soil reaction below pH 5.5 produced turf of low vigor. Injury from deficient moisture was first visible on acid soils, the response from new applications of fertilizer was less marked than on comparable plots with higher pH values, and growth ceased earlier in the fall and began later in the spring on the more acid soils. Although such behavior may be attributed wholly or in part to factors unrelated to root development, nevertheless the danger of using root growth as an index of the favorableness of a given treatment is quite obvious. If it were possible readily to separate living functional roots from dead or non-functional ones, yield of roots would be a more valuable criterion. On newly seeded areas the entire root system may be assumed to be entirely alive and functional. The roots beneath established sod must always be composed of both living and dead roots, and the amount of dead roots will be diminished as conditions become more favorable for their rapid decay.

It is clear from the results in table 6 that the effect of the available phosphorous supply may be wholly masked by the reaction factor. Thus, ammonium phosphate treatments which more than doubled the supply of available phosphate induced no greater root growth than did ammonium sulfate. From these findings, it seems necessary to investigate possible differences in soil reaction as the explanation for greater root abundance accompanying applications of ammonium phosphate in such cases as that noted by Evans (1), before concluding that increased phosphorous supply has enhanced root growth.

The evidence from these root harvests does not indicate that the phosphorous supply has influenced root development when the soil reaction is favorable for top growth. Alfalfa meal and bone meal produced almost identical root growth in spite of the fact that there were only 58 p.p.m. of available phosphorous for alfalfa meal plots as compared with 392 for bone meal plots. There is a possibility that the supply of this element in the unfertilized soil was adequate to satisfy the needs of the grass, and that further applications therefore produced no stimulating effect. No data are available regarding the phosphate requirements of the species.

A positive correlation of minor magnitude may be noted between root abundance and organic carbon content of the soil. Variation in carbon was small and may have been caused entirely by fine root fragments which could not be readily separated from the soil by screening. The minor changes in carbon content apparently bore no causal relation to root development.

There is some evidence that the provision of nitrogen in a readily available form tends to restrict root growth as compared with application of nitrogen in a slowly available form. The 5–10–5 fertilizer carried two-thirds of its nitrogen in the form of sulfate of ammonia and nitrate of soda which are immediately available, whereas alfalfa meal and bone meal release nitrogen gradually as the materials decay. The supply of soluble nitrogen in early spring when roots are being formed is doubtless considerably smaller on plots receiving the organic materials than on those receiving the 5–10–5 mixture. Since a high nitrogen-carbohydrate ratio has been noted by various workers to stimulate top growth rather than root growth, the difference between root weight below the first inch of 9.8 pounds for the 5–10–5 fertilizer and 18.5 and 20.2 respectively for the two organic materials, may be attributed to the quantity of soluble nitrogen supplied during the spring months when the roots are being formed. The evidence is not sufficiently conclusive, however, to establish the principle on turf grasses. The possibility of such a relation is worthy of further consideration, since the early application of sulfate of ammonia to pastures and other turf for the purpose of inducing early spring growth may prevent vigorous root growth and thus increase the injury from drought in summer when maintenance of vegetative activity is of greatest importance.

The correlation of root growth with soil characteristics at different soil levels is shown for three types of treatment in table 7. In one case, no fertilizer had been applied during the 5-year period of observation, whereas on the other plots nitrogen had been supplied regularly in the form of ammonium nitrate. Flowers of sulfur was added to increase the soil acidity on several of the fertilized plots, and hydrated lime was added to an equal number. The sulfur treatments reduced the pH values below 5.0 in the first 4 inches and increased the acidity to a lesser extent to the depth of 10 inches. The lime treatment was equally effective in changing the reaction, with the result that at the end of 5 years the reaction of all layers was above neutrality. The change in reaction to neutrality or alkalinity was accompanied by an increase in supply of available phosphorous. There was no significant response in content of organic carbon to the change in reaction of the soil layers. Root abundance was not positively correlated with either the supply of available phosphorus or the organic carbon content. Top growth proved greatly superior, however, on the limed plots, which were not only richer in quantity of available phosphorous but also were neutral in reaction. Either the roots on the limed soil were more effective per unit of weight in utilization of the soil resources, or a very high percentage of the roots on the sulfur plots were dead, leaving a smaller amount of functional roots in the acid soil than in the limed soil.

TABLE 7

Relation of soil conditions at different levels, to root development, with different treatments. Virginia creeping bent, fertilized since July 1926. Pounds of roots under 1,000 square feet of surface harvested September 1–8, 1931*

SOIL LAYER	NO FERTILIZER				AMMONIUM NITRATE AND SULFUR				AMMONIUM NITRATE AND LIME			
	Weight of roots	Soil reaction	Available phosphorus	Organic carbon	Weight of roots	Soil reaction	Available phosphorus	Organic carbon	Weight of roots	Soil reaction	Available phosphorus	Organic carbon
	lbs.	*pH*	*p.p.m.*	*per cent*	*lbs.*	*pH*	*p.p.m.*	*per cent*	*lbs.*	*pH*	*p.p.m.*	*per cent*
1st inch†	39.3	5.9	35	2.5	78.7	4.8	25	2.5	20.6	7.7	74	2.6
2nd inch	12.3	5.8	45	2.1	79.3	4.9	24	2.4	4.2	7.8	89	2.0
3rd inch	2.7	5.8	50	1.9	14.5	4.8	58	2.0	0.8	7.9	105	1.8
4th inch	2.0	5.8	57	2.0	6.6	5.0	68	1.8	1.3	7.8	104	1.7
5th inch	1.3	5.9	62	1.9	6.6	5.4	70	1.7	1.1	7.8	104	1.7
6th inch	0.7	6.0	79	1.8	4.8	5.5	73	1.7	0.4	7.6	106	1.8
7th inch	0.7	6.1	73	1.8	3.6	5.6	69	1.6	0.3	7.5	109	1.6
8th inch	0.4	6.1	68	1.8	1.7	5.6	68	1.5	0.3	7.3	97	1.6
9th inch	0.6	6.1	66	1.7	1.1	5.7	68	1.5	0.1	7.0	93	1.7
10th inch	0.3	6.0	69	1.7	0.4	5.7	56	1.6	0.1	7.0	83	1.8
Total roots...	60.3	.....	.....	.....	197.3	.....	.....	.....	29.2	.....	.....	.....
Roots below 1st inch....	21.0	.....	.....	.....	118.6	.....	.....	.....	8.6	.....	.....	.....

* Fertilizers applied in four to five equal parts during the season to supply 2.83 pounds nitrogen per 1,000 square feet of surface. Sulfur applied at rate of 13.8 pounds per 1,000 square feet each season, and hydrated lime at the rate of 137.8 pounds per 1,000 square feet each season.

† Root weights in the 1st inch are *not* corrected for presence of underground stems.

An excellent opportunity for the comparison of root growth under conditions of similar reaction but of different phosphorous supply is given in table 8. The plots receiving nitrate of soda and a complete 5–10–5 fertilizer respectively were nearly identical in respect to soil acidity with the exception of the upper 3 inches. Both plots received equal amounts of nitrogen, but in the case of the complete 5–10–5 fertilizer the nitrogen supplied was in the form of one-third tankage, one-third sulfate of ammonia, and one-third nitrate of soda. The difference in reaction of the upper soil layers of the sodium nitrate and 5–10–5 plots may be attributed to the sulfate of ammonia in the complete fertilizer which increased acidity. The phosphorus content of the soil layers in the 5–10–5 plots was several times as great as in the nitrate of soda plots throughout most of the layers.

In spite of striking differences in phosphorous content, there was little or no change in root abundance. The minor increase in quantity of roots noted in the upper 4 inches of the 5–10–5 plots as compared with nitrate of soda plots may properly be attributed to the higher acidity of these layers, since no increase in root abundance occurred in the layers below the fourth inch. As observed previously, there was no significant positive relation between either quantity of available phosphorous or organic carbon content of the soil layers and root growth. Such increases in root development as may be observed, accompanied increased acidity. Since higher soil acidity was correlated with poorer top growth, there is justification for the belief that many of the roots in the acid soil were either dead or non-functional, and that root abundance is not an adequate measure of the favorableness of a given treatment to root development unless soil reaction is of the same order of magnitude. The prin-

TABLE 8

Relation of soil conditions at different levels, to root development, with different fertilizers. Virginia creeping bent, fertilized since July 1926. Pounds of roots under 1,000 square feet of surface harvested September 1–8, 1931*

SOIL LAYER	NITRATE OF SODA				SULFATE OF AMMONIA				5–10–5 FERTILIZER			
	Weight of roots	Soil reaction	Available phosphorus	Organic carbon	Weight of roots	Soil reaction	Available phosphorus	Organic carbon	Weight of roots	Soil reaction	Available phosphorus	Organic carbon
	lbs.	*pH*	*p.p.m.*	*per cent*	*lbs.*	*pH*	*p.p.m.*	*per cent*	*lbs.*	*pH*	*p.p.m.*	*per cent*
1st inch†	36.7	6.2	56	2.7	74.3	5.3	25	2.7	42.6	5.6	239	2.7
2nd inch	3.2	6.3	53	2.2	23.7	4.9	26	2.6	4.5	5.8	209	2.3
3rd inch	2.5	6.3	63	1.9	12.1	4.9	47	2.1	2.7	6.0	234	1.9
4th inch	1.3	6.2	65	1.8	3.8	5.2	60	2.0	0.8	6.2	228	2.0
5th inch	0.4	6.2	68	1.8	2.4	5.3	62	1.9	0.6	6.4	171	1.8
6th inch	0.4	6.3	73	1.6	1.4	5.4	59	1.7	0.4	6.5	149	1.7
7th inch	0.4	6.3	103	1.7	2.2	5.5	60	1.8	0.3	6.6	127	1.7
8th inch	0.4	6.4	84	1.6	1.3	5.6	60	1.8	0.3	6.5	106	1.7
9th inch	0.1	6.4	67	1.5	1.1	5.8	61	1.7	0.1	6.5	68	1.6
10th inch	0.1	6.3	55	1.5	0.3	6.0	54	1.7	0.1	6.3	64	1.5
Total roots...	45.5	.....	.....	.....	122.6	.....	.....	.....	52.4	.....	.....	.....
Roots below 1st inch....	8.8	.....	.....	.....	48.3	.....	.....	.....	9.8	.....	.....	.....

* All plots received 2.83 pounds nitrogen per 1,000 square feet per year.

† Root weights in the 1st inch are *not* corrected for presence of underground stems.

cipal factor or factors limiting root occupation of the deeper soil layers of the soil type on which these experiments were conducted have not been identified. Aeration and physical resistance to penetration are two unmeasured factors which will merit consideration.

Further comparisons of root development under differing fertilizer treatments are given in table 9. The grass species in these tests was the metropolitan strain of creeping bent, but the general treatment given and the soil conditions are similar to those on the close-clipped plots discussed in the preceding paragraphs. In general the results are similar to those noted in table 6. Sulfate of ammonia, which produced a reaction of pH 5.3 in the upper 4 inches, was responsible for heavy accumulations of roots. Urea applied as a fertilizer at the rate yielding 2.83 pounds of nitrogen per 1,000 square feet yearly, reduced the pH value of the soil to 5.6. Since there was no unusual accumulation of roots at this reaction, it may be concluded that the critical point lies between pH 5.6 and 5.3 for this particular soil.

TABLE 9

Weight of roots of Metropolitan creeping bent, at different soil levels, in pounds under 1,000 square feet of surface, as affected by various fertilizer treatments. Fertilized regularly since July, 1926; roots harvested September 1–8, 1931*

SOIL LAYER	WEIGHT OF ROOTS UNDER 1,000 SQUARE FEET			
	No fertilizer	Sulfate of ammonia	Urea	Cottonseed meal
	lbs.	*lbs.*	*lbs.*	*lbs.*
1st inch†	68 0	151.2	105.5	70.0
2nd inch	10.0	90.0	22.0	25.1
3rd inch	3.2	25.1	4.1	4.9
4th inch	1.0	4.1	0.7	1.1
5th inch	1.0	2.0	0.6	0.7
6th inch	0.4	0.8	0.4	0.4
7th inch	0.3	0.7	0.4	0.3
8th inch	0.1	0.3	0.4	0.3
9th inch	0.1	0.3	0.3	0.1
10th inch	0.3	0.1	0.1	0.1
Total roots	84.4	274.6	134.5	103.0
Roots below 1st inch	16.4	123.4	29.0	33.0

Average conditions in upper 4 inches of soil

Soil reaction, pH	5.8	5.3	5.6	6.1
Available phosphorus, p.p.m.	52.0	40.0	35.0	53.0
Organic carbon, per cent	1.9	2.1	2.0	1.9
Vigor of turf	poor	medium	med. to good	good

* All fertilizers were applied in four or five equal parts during the season, at rates to supply 2.83 pounds nitrogen per 1,000 square feet per year.

† Weights of roots in first inch are *not* corrected for presence of underground stems.

The unfavorable effect of soil acidity on top growth did not become apparent on these plots and on those indicated in table 6, until the pH values fell to approximately 5.5. Likewise, heavy root accumulations did not occur until this degree of acidity was attained, suggesting that the two phenomena are related. In any event, pH values that permit increased abundance of roots also affect top growth adversely. It seems likely that soil reactions which do not permit ready decomposition of dead roots also inhibit the release of plant nutrients from the soil. The accumulation of roots may directly hamper top growth by preventing the ready entrance of moisture to the soil, a factor of some importance in dry periods or in seasons with torrential rains. Strong acidity doubtless affected these turf plants directly as well as indirectly, but it is obvious from these observations that the increased root yields found on strongly acid soils are not associated with any greater ability of the plants to utilize the current soil resources.

There was no obvious correlation between the supply of available phosphorous and root growth or between the organic carbon content of the soil and development of roots. As in the previous series of plots, the factor or factors stimulating increased growth of grass roots on these soils were not isolated. If organic carbon content and available phosphorous are of any importance, their effect is masked by the unidentified factor or factors. The possibility of soil ventilation or physical resistance of the soil to root penetration being of prime importance in their effect on root occupation of the podzolic soils of the eastern United States warrants careful investigations.

SUMMARY

An original method for determining abundance of roots for pasture and turf grasses was devised to permit a study of root development on a typical podzolic soil of the gray-brown forest soil group.

Periodic harvest of roots on Kentucky bluegrass and colonial bent grass during the months of April, May, and June indicated that bluegrass began formation of new roots approximately 2 weeks earlier than bent grass, apparently because of a difference in the minimum temperatures at which growth could proceed. New roots were produced on bluegrass until May 4, whereas formation of new roots on colonial bent continued intermittently until about June 15.

The maximum root weight for bluegrass occurred in early May and practically 2 weeks later for bent grass. The maximum root weight was approximately twice as great as the quantity present at the beginning of the season, indicating that at least one-half of the root system is newly generated each spring. This being the case, soil and cultural conditions in early spring are more likely to affect root development than at other seasons. Practically all of the roots for both species were found in the upper 9 inches of soil on the soil type investigated, the abundance decreasing rapidly with depth.

The subterranean growth in the first inch of soil always consisted of stems and roots. Since total root weight was highly correlated with root weight below the first inch, the latter value may be relied upon for comparative root studies, thus eliminating the necessity for separating roots and stems in the upper soil level.

A detailed comparison of the root development of six grass species in June 1931 showed hard fescue to be the only grass with roots extending in significant quantity below the depth at which the soil had been plowed in previous cropping. There was a rapid decline in the abundance of roots of all species between the first and sixth inch soil layers. This reduction was apparently not caused by changes in soil reaction, available phosphorous supply, or quantity of soil organic matter as measured by organic carbon content. In general, reduced root development below the sixth inch was associated with greater soil acidity, lower available phosphorous, and lower carbon content, but the relation was apparently not one of cause and effect.

Mowing twice weekly at a height of ¼-inch greatly restricted root development of colonial, seaside, and velvet bent grasses, as compared with ⅞-inch with the effect most pronounced from the third inch downward. Although hard fescue possessed more abundant root growth than the three bent species, when cut at ¼-inch, this characteristic did not prevent eventual killing of the fescue. The death of hard fescue under close mowing was apparently a case of gradual starvation caused by continuous and severe defoliation made possible by the erect habit of growth of the tops.

The root development of Kentucky bluegrass clipped at ⅞-inch twice weekly was fully as abundant as that of uncut bluegrass. Root development of redtop was reduced by regular mowing at ⅞-inch. From the behavior of the two species bluegrass appears better suited than redtop to the repeated clipping occurring on pastures and lawns.

Although the three bent species tolerated regular mowing at a height of ¼-inch, the ratio of roots to tops produced was considerably lower than when mowed at a ⅞-inch length. The longer cut favored relatively greater occupation of the deeper soil layers, and therefore greater opportunity to maintain growth in periods of adversity. The ratio of roots to top was comparatively low for bluegrass, not because of scanty root development but because of abundant top growth. The low root-top ratio of this species was compensated for by the underground placement of the creeping stems and the ability to become dormant in unfavorable seasons.

The root development of creeping bent grass cut at ¼-inch length with 12 different systems of fertilization indicated that the residual effect of fertilizers on the soil is of great importance. Total root weights in the upper 10 inches of soil were several times as great on plots with pH values below 5.5 as on those with reactions of pH 5.8 or above. Since the vigor of tops was inversely correlated with root abundance, and many of the grass roots in the strongly acid soils appeared to be dead, the conclusion is drawn that the roots produced in previous years had accumulated because the soil reaction was not favorable for their decay.

Comparisons of grass root systems under different types of management should not be used to measure the effect of the treatment on root development, unless the soil reaction has permitted decay of dead roots.

A lack of correlation between the supply of available phosphorous, organic carbon content, or abundance of lime, and root development suggests that certain unidentified factors control root development and occupation of soil layers on such soil types. Aeration and physical resistance to root penetration are suggested as possible factors that would merit consideration.

Slowly available nitrogenous fertilizers permitted somewhat greater root development than soluble fertilizers supplying equal amounts of total nitrogen. Since relatively high ratios of soluble nitrogen to carbohydrate reserves have been shown to favor top growth rather than root development, it seems possible that early spring applications of available nitrogeneous fertilizers in abundance may reduce the quantity of new roots formed and thus limit utilization of the soil resources for the remainder of the season.

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SOLUBLE ALUMINUM STUDIES: IV. THE EFFECTS OF PHOSPHORUS IN REDUCING THE DETRIMENTAL EFFECTS OF SOIL ACIDITY ON PLANT GROWTH[1]

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The work of various investigators (1, 4, 5, 7, 13) has definitely established the fact that the addition of large amounts of superphosphate to very acid soils will reduce, at least temporarily, the detrimental effects of these soils on the growth of plants. Although it is held by some that this is due either to the reduction in the acidity of the soil or to the supplying of the phosphate needs of the crop (9), many investigators believe that the main action of large amounts of phosphate lies in correcting the toxicity of aluminum found in these acid soils. There is no agreement, however, as to the manner in which phosphates render aluminum nontoxic. Since aluminum phosphate is much more insoluble than most other compounds of aluminum, the majority of workers are inclined to believe that the addition of large amounts of phosphate to acid soils precipitates the soluble aluminum present (1, 2, 5, 7, 13). This conclusion is based partly on the results of the investigations of Burgess and Pember (3) and of Blair and Prince (2) which showed that the amount of so-called "active aluminum" determined by means of 0.5 *N* acetic acid is decreased by the addition of large amounts of phosphate. The significance of the amount of aluminum extracted from soils by such a strong reagent, however, has been seriously questioned, and no data have been reported regarding the effect of phosphates on the amount of aluminum found in the soil solution.

Other workers believe that the beneficial action of large amounts of phosphate in correcting aluminum toxicity takes place within the plant. Burgess and Pember (3) found that plants grown on an acid, untreated soil contained as much aluminum as plants grown on the same soil supplied with large amounts of superphosphate. The latter plants, however, contained three to five times as much phosphorus. As a result of these studies they state:

> The results of these chemical studies taken by and large indicate that while heavy soluble phosphate applications to acid soils do, to a considerable degree, directly precipitate the active aluminum which may be contained therein, its most important function is performed within the plants themselves where a certain fraction of the absorbed phosphorus acts as an antidote and is, in turn, rendered unavailable for normal nutritional purposes.

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McGeorge (10) arrived at a similar conclusion as a result of his studies, in which he found that if a part of the roots of a plant were placed in a solution high in phosphorus while the rest were in an aluminum solution, the roots in the former grew normally, and top growth was normal.

On the other hand the more recent work of McLean and Gilbert (12) with culture solutions indicates that the beneficial effect of phosphate lies in precipitating the aluminium in the solution. Soluble phosphates added to culture solutions in quantities equivalent to that of aluminum counteracted the toxicity of the latter, presumably because of the precipitation of aluminum as phosphate. In another experiment in which the precipitation of aluminum phosphate was prevented by alternating the plants between cultures containing aluminum and others containing two different concentrations of phosphate, they found no significant difference in favor of the cultures receiving the high amounts of phosphorus. The recent works of Parker (14) and of Tidmore (19) on the optimum concentrations of phosphate in culture solutions suggest, however, that the lower amounts of phosphate used by McLean and Gilbert, namely, 30 p.p.m., might have been high enough to reduce the toxicity of aluminum and that higher concentrations of phosphate would therefore be of no further value. Moreover, the high amounts of phosphorus used in the experiment apparently depressed the yields of rye and corn.

It is thus apparent from a review of the literature that there is no unanimity of opinion nor much conclusive data regarding the reason for the beneficial action of high amounts of phosphate on acid soils. In view of the general interest regarding the questions of phosphate fertilization and soil acidity it seemed desirable to make a further study of this problem.

The objectives of this study are as follows:

To determine the effect of adding different kinds and amounts of phosphate compounds on the H-ion concentration and on the aluminum, calcium, and phosphate concentrations of the soil solution.

To study the relation between the aluminum concentration of the soil solution as affected by phosphate fertilization and the growth of plants.

To determine the effect of sub-optimum concentration and of high concentration of phosphate in culture solutions on the toxicity of aluminum to Cos lettuce.

To determine the effect of aluminum in culture solutions on the concentration of phosphorus in the plant sap of lettuce.

EXPERIMENTAL RESULTS

The effect of phosphate fertilization on the aluminum concentration of the soil solution and on the growth of barley and alfalfa

In order to study the effect of different kinds and amounts of phosphate compounds on the concentration of aluminum in the soil solution and on the growth of plants, 22 two-gallon pots were each filled with 8,000 gm. of a Wheeling fine sandy loam of pH 4.60. Fertilizers and lime in the amounts shown in table 1 were thoroughly mixed with the soil and the pots planted to barley on

October 21. Since it was desired to study the effect of the treatment on the soils at various intervals, 1-gallon pots containing one-half as much soil each were also given similar treatments. Samples of soil were removed from the

TABLE 1

*Treatment of soil cultures**

POT NUMBER	PHOSPHATE FERTILIZATION		AMOUNTS OF CALCIUM CARBONATE PER ACRE
	Source of phosphate	Amount of P_2O_5 per acre	
		lbs.	*lbs.*
1–2	....	.....	.....
3–4	....	.....	3,250
5–6	Superphosphate (20 per cent)	100	625
7–8	Superphosphate (20 per cent)	100	3,250
9–10	Superphosphate (20 per cent)	100	.....
11–12	Superphosphate (20 per cent)	500	.....
13–14	Superphosphate (20 per cent)	2,000	.....
15–16	Monocalcium phosphate	2,000	.....
17–18	Monosodium phosphate	100	.....
19–20	Monosodium phosphate	500	.....
21–22	Monosodium phosphate	2,000	.....

* All cultures received KNO_3 and NH_4NO_3, each at the rate of 100 pounds per acre, 2,000,000 pounds of soil.

TABLE 2

The phosphate and hydrogen-ion concentration of soil extracts at different dates as affected by the various soil treatments

POT NUMBER	H-ION CONCENTRATION				PO_4 CONCENTRATION (EXTRACT BASIS)			
	11/11/30	12/17/30	1/14/31	Average	11/11/30	12/17/30	1/14/31	Average
	pH	*pH*	*pH*	*pH*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1–2	4.68	4.50	4.58	4.59	tr*	tr.	tr.	tr.
3–4	6.53	6.45	6.43	6.47	tr.	tr.	tr.	tr.
5–6	4.98	4 85	5.03	4.95	tr.	tr.	tr.	tr.
7–8	6.48	6.30	6.35	6.38	0.08	tr.	tr.	tr.
9–10	4.63	4.50	4.55	4.56	0.04	0.04	tr.	tr.
11–12	4.63	4.50	4.60	4.58	0.21	0.15	0.21	0.19
13–14	4.65	4.60	4.65	4.63	5.88	3.25	4.00	4.38
15–16	4.85	4.85	4.90	4.87	9.13	6.50	6.50	7.38
17–18	4.70	4.65	4.78	4.71	0.09	0.06	0.13	0.09
19–20	4.95	4.85	5.10	4.97	0.91	0.68	0.90	0.83
21–22	5.65	5.50	5.58	5.58	32.00	24.00	27.00	27.67

* tr. = trace.

1-gallon pots for determinations of pH, water-soluble phosphorus, and conductivity on both November 11 and December 17. At the latter date the displaced soil solutions were also obtained for studies of aluminum, phosphate, calcium, and H-ion concentrations. Immediately after the barley was harvested on January 14, the displaced soil solutions were obtained from the soil in the 2-gallon pots for similar studies. The results of the analyses are given in tables 2 and 3, and the yields of barley are also given in table 3.

It will be noted from the data in table 2 that superphosphate, even at the highest rate of application (5 tons per acre) had very little effect on the hydrogen-ion concentration of this soil. A similar amount of phosphorus in monocalcium phosphate, however, raised the pH from about 4.60 to 4.85. Sodium phosphate, especially at the two higher rates of application raised the pH value of the soil considerably.

The effects of the changes in the hydrogen-ion concentration of the soil on the concentrations of aluminum in the soil solution are readily seen in table 3.

TABLE 3

Soil solution data and yield of barley

POT NUMBER	H-ION CONCENTRATION b	ANALYSES OF DISPLACED SOLUTIONS						YIELD OF BARLEY [OVEN-DRY WEIGHT]
		Calcium		Aluminium		PO$_4$	Sp. Res.	
		a*	b	a	b	b	25°C. b	
	pH	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*ohms*	*gm.*
1–2	4.58	392	297	17.8	15.8	tr.	243	0.71
3–4	6.43	1,022	562	None	None	tr.	187	5.15
5–6	5.03	816	338	3.1	1.2	tr.	198	4.90
7–8	6.35	1,242	595	None	None	tr.	165	7.05
9–10	4.55	640	710	15.2	13.5	0.25	194	1.28
11–12	4.60	966	369	14.6	10.8	0.13	172	5.90
13–14	4.65	1,028	816	10.1	8.7	1.53	172	9.95
15–16	4.90	657	384	None	None	3.75	243	9.70
17–18	4.78	125	62	5.3	2.5	0.14	243	4.60
19–20	5.10	73	36	1.0	0.5	0.16	175	8.00
21–22	5.58	25	30	None	None	26.00	148	4.25

* a = soil solutions displaced on November 11; b = soil solutions displaced on January 14.

No aluminum was found in the displaced soil solutions where large amounts of lime had been added. This was also true where large amounts of calcium and sodium phosphates had been applied. In the latter cases, however, the precipitation of aluminum was apparently a result of both the increased pH value and the increased phosphate concentration of the soil solution.

Although the use of superphosphate decreased the concentration of aluminum in the soil solution, between 8.7 and 10.1 p.p.m. still remained in solution in the soil which had received the highest application of superphosphate. This is especially significant when the yield data are considered, for it will be noted that the highest yield of barley was obtained with this treatment in spite of the fact that 8 to 10 p.p.m. of aluminum were found in the soil solution. Moreover, pots 11 and 12, which received the medium application of superphosphate per acre and still contained 10.8 to 14.6 p.p.m. of aluminum in the displaced soil solution, yielded slightly more than pots 3 and 4, which received 3,250 pounds of calcium carbonate per acre and contained no aluminum in the soil solution. In view of the fact that 1 p.p.m. of aluminum was found decidedly injurious to barley in culture solutions in a previous experiment (8) these results indicate that the beneficial effect of large amounts of superphosphate on acid soils is not due necessarily to the precipitation of aluminum in the soil solution, but may also be due to the reduction of the injurious action of aluminum in the plant. It may be possible that the high concentration of calcium in the soil solution of pots 13 and 14 is partly responsible for the good growth in spite of the presence of considerable soluble aluminum. When it is considered, however, that the concentration of calcium is noticeably high in cultures 1 to 16 and that cultures 5 and 6 made relatively poor growth in the presence of only small amounts of aluminum, it would seem that the phosphorus plays the important rôle.

The use of large amounts of sodium phosphate was detrimental to the growth of barley. This was probably due to the high concentration of sodium as compared with calcium ions in solution. With medium amounts of sodium phosphate, however, good growth of barley was obtained even though only 36 to 73 p.p.m. of calcium were found in the displaced soil solution. Most of the calcium apparently was precipitated as calcuim phosphate as a result of the high concentration of phosphate in solution.

After the barley roots were removed from the soil, potassium nitrate and ammonium nitrate were again added to all pots each at the rate of 100 pounds per acre. No additional phosphate fertilizers were applied. Wheat was planted in the pots but since it made a very poor growth as a result of the lack of sunshine, the young plants were removed, and alfalfa was sown in these pots on February 10. The early growth of alfalfa was poor in all pots except those which received lime and those which received the three forms of phosphate at the highest rate (table 4). The growth in the latter was practically as good as in the best pots, nos. 7 and 8. After about 3 months the growth in the six pots which received heavy phosphate fertilization, however, became very slow (the plants becoming paler green in color), whereas the plants which had made little growth at first continued to grow slowly and kept a green color. These latter differences were also apparent in the new growth after the first cutting which was made on June 13. Since these soils were very acid, it was suspected that symbiotic nitrogen fixation was not taking place and that the failure of the plants in pots 13 to 16 to continue to make good growth was due to a lack of nitrogen. The good early growth on these pots was made at the expense of the nitrates left in the soil from the fertilization of the wheat. Ammonium nitrate at the rate of 100 pounds per acre was therefore added about 2 weeks after the first cutting to those pots which had received phosphate fertilizers at the rate of 100 and 2,000 pounds P_2O_5 per acre without lime. Soon after the addition of nitrogen the plants became greener, and good growth took place in those pots which had received the heavy phosphate fertilization. A similar nitrogen application was made on August 13, about 2 weeks after the second cutting. The soil solutions were displaced on September 24, immediately after the last cutting was made.

The yields and the analytical data on the soil solution are given in table 4. As will be noted, the results are much in agreement with those obtained with barley. The most significant point is that good growth of alfalfa was made on pots 13 and 14, which had received large amounts of superphosphate, even though the soil contained about 8.00 p.p.m. of aluminum in solution and had a pH of 4.58. In order to obtain this good growth, however, the addition of nitrogen was necessary as is shown by the fact that the growth of alfalfa was better in pots 9, 10, 17, and 18 than in pots 11, 12, 19, and 20. The former pots had received nitrogen fertilizers whereas the latter had not. Even though some nodules were present on the roots, nitrogen fixation was apparently not taking place fast enough in these acid soils to furnish the plant with the necessary nitrogen. This is in agreement with the conclusions of Sewell and Gainey (18) that the poor growth of alfalfa on acid soils may be due more to the effect of acidity on symbiotic nitrogen fixation than on the immediate growth of the plant.

TABLE 4

The yield of alfalfa as related to pH and to the aluminum and phosphate concentrations of the soil solution

POT NUMBER	H-ION CONCENTRATION	PO_4 IN SOIL EXTRACT (DRY SOIL BASIS)	Al CONCENTRATION IN SOIL SOLUTION	YIELD* OF ALFALFA PER POT [OVEN-DRY WEIGHT]			
				1st cutting	2nd cutting	3rd cutting	Total
	pH	*p.p.m.*	*p.p.m.*	*gm.*	*gm.*	*gm.*	*gm.*
1–2	4.73	0.22	2.88	1.50	5.35	3.75	10.60
3–4	5.88	0.27	N.D.	6.20	8.50	6.40	21.10
5–6	4.90	0.25	Trace	4.25	2.35	1.65	8.25
7–8	5.88	0.26	None	6.90	7.90	6.95	21.75
9–10	4.60	0.53	6.25	4.30	4.50	3.30	12.10
11–12	4.55	0.61	11.75	4.40	2.50	1.75	8.65
13–14	4.58	5.63	8.00	4.80	7.25	5.25	17.30
15–16	4.50	5.63	N.D.	4.45	5.60	5.80	15.85
17–18	4.58	0.26	3.00	3.20	3.60	2.85	9.65
19–20	4.63	1.63	2.63	3.80	1.80	1.55	7.15
21–22	5.58	50.65	None	5.90	4.70	4.95	15.45

* The first cutting was removed on June 13, the second on July 27, and the third on September 23.

From these results with barley and alfalfa it would appear that the beneficial action of large amounts of phosphate cannot be entirely explained by the precipitating action of the phosphate on the aluminum in solution. Although 8 to 10 p.p.m. of aluminum remained in the soil solution after heavy fertilization with superphosphate, good growth of barley and alfalfa was obtained. Since poor growth of crops has been obtained in acid soils containing less than 2 p.p.m. of aluminum in the soil solution (15) and since in culture solutions barley was decidedly injured by 1.0 p.p.m. of aluminum (8), it seems evident that the beneficial action of the large amounts of superphosphate was due to the effect of the phosphorus in correcting the toxic action of aluminum in the plants.

Effects of different amounts of phosphorus on the yield and composition of Cos lettuce in culture solution

Culture solutions were used for a further study of the effect of phosphorus in reducing the injurious action of aluminum on plant growth. Cos lettuce was chosen as the test plant because it has been shown to be sensitive to aluminum (11). The seeds were germinated in quartz sand and the seedlings transferred to the culture solutions when about 12 days old. In general, the culture solution technic was very similar to that used in a former investigation (8). The culture vessels were of 15-liter capacity and a modified Hartwell and Pember's nutrient solution (8) made up with distilled water was used.

TABLE 5

The effect of aluminum on the yield of Cos lettuce grown in culture solutions of different phosphate concentrations

CULTURE NUMBER	CULTURE SOLUTION DATA				YIELD OF LETTUCE (OVEN-DRY WEIGHT)*				
	PO_4	H-ion concentration		Al	Tops	Roots	Total	Relative†	$\frac{\text{Top}}{\text{Roots}}$
		Desired	Found						
	p.p.m.	*pH*	*pH*	*p.p.m.*	*gm.*	*gm.*	*gm.*		
1A, 1C, 8B, 8D.........	1	6.00	5.46	None	3.91	0.33	4.24	146.2	11.9
2A, 2C, 9B, 9D.........	1	4.50	4.40	None	2.62	0.28	2.90	100.0	9.3
3A, 3C, 10B, 10D.......	1	4.50	4.43	1.0	2.14	0.26	2.40	82.7	8.2
4A, 4C, 11B, 11D.......	1	4.50	4.47	2.5	0.73	0.11	0.84	29.0	6.4
5A, 5C, 12B, 12D.......	50	4.50	4.34	None	8.20	0.50	8.70	100.0	16.5
6A, 6C, 13B, 13D.......	50	4.50	4.30	1.0	6.98	0.41	7.39	85.0	17.0
7A, 7C, 14B, 14D.......	50	4.50	4.35	2.5	6.27	0.42	6.69	76.9	15.1

* Average weight per plant. Cultures were in quadruplicate, with three plants in each culture in low phosphate series and two plants per culture in high phosphate series.

† In the low-phosphate series, culture 2 was taken as 100, whereas in the high-phosphate series culture 5 was taken as 100.

The treatments of the cultures with respect to phosphate, aluminum, and hydrogen-ion concentration are given in the first few columns of table 5. For convenience the cultures receiving 1.0 p.p.m. phosphate will be referred to as the low-phosphate series and those receiving 50 p.p.m. phosphate as the high-phosphate series. In each series certain cultures received aluminum in concentrations of 0, 1.0, and 2.5 p.p.m. In order to keep both the aluminum and the phosphate in solution these two constituents were not added to the nutrient solution at the same time, but the plants were alternated daily between cultures receiving the nutrient solution containing phosphorus and similar cultures without phosphate receiving from 0 to 2.5 p.p.m. of aluminum. The roots of the plants were always washed in distilled water when the transfer was being made. The pH of the solutions was kept at 4.5 except cultures 1 and 8, which served as a check on the effect of pH on growth. The phosphate was added as monosodium phosphate and the aluminum as aluminum chloride. Iron was supplied to the plants in separate culture vessels about once a week (8). Fresh culture solutions were made up every 2 weeks, and each culture was aerated for about 5 minutes daily.

The hydrogen-ion concentration of the solutions was adjusted to the original reaction daily. Phosphate determinations of the low phosphate series were made three times a week and the required amount of phosphate was added to maintain the desired concentration. On days that the phosphate concentration was not checked, an amount of phosphate was added equal to one-half that absorbed during the previous 2-day period. The phosphate concentration of the high-phosphate series was renewed only every 2 weeks when the new solutions were made up. The aluminum concentration of the culture solutions was determined twice a week during the first part of the experiment, but after only slight variations from the desired concentrations were found the determinations were made weekly.

The effect of the two concentrations of aluminum used was evident in the plants of the low-phosphate series in the early stages of growth in both tops and roots. The latter showed the characteristic aluminum injury previously described (8). A slight injury to the roots of the plants of the high-phosphate series was also noted in the early stages of growth where 2.5 p.p.m. of aluminum was present.

The appearance of some of the plants on November 20 is shown in figures 1 and 2 of plate 1, and of some on December 11 in figures 3 and 4 of plate 1. The plants were harvested immediately after the latter photographs were taken.[3] The average weights and also the relative weights based on the "no aluminum" cultures of each series as 100 are given in table 5. It will be noted that the presence of aluminum resulted in reduced growth of roots and tops in both the high- and low-phosphate series. The percentage reduction in yields from the high concentration of aluminum, however, is much greater in the latter than in the former. In the high-phosphate series 2.5 p.p.m. of aluminum caused a reduction in yield of only about 23 per cent, whereas in the low-phosphate series the reduction in yield was 71 per cent. These data, therefore, support the results obtained with the soil cultures, namely, that the presence of large amounts of phosphate lessens the injurious action of aluminum on plant growth in other ways than by precipitating aluminum from solution, since in this experiment such a precipitation could not take place.

The plant analytical data given in table 6 also support this conclusion. It will be noted that the plants of the high-phosphate series contained as high a percentage of aluminum in the tops as the corresponding plants of the low-phosphate series in spite of the much smaller injury from aluminum in the former. The plants which received 50 p.p.m. of phosphate, however, had about twice as high a percentage of phosphorus as those which had received only 1.0 p.p.m. of phosphate. These results are in agreement with those of Burgess and Pember (3) and lend support to the theory that large amounts of phosphate function at least partially by reducing the toxic effect of aluminum within the plant.

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[3] Sap samples were obtained from these plants for further studies, which will be discussed later.

TABLE 6

The phosphate and aluminum content of lettuce grown in culture solutions of different phosphate and aluminum concentration

CULTURE NUMBER	CULTURE SOLUTIONS			COMPOSITION OF LETTUCE			
	PO_4	Al	H-ion concentration	Tops		Roots*	
				PO_4	Al	PO_4	Al
	p.p.m.	*p.p.m.*	*pH*	*per cent*	*per cent*	*per cent*	*per cent*
1 and 8	1.0	None	6.0	0.784	.0264	.....	.....
2 and 9	1.0	None	4.5	0.786	.0286	.....	.....
3 and 10	1.0	1.0	4.5	0.700	.0282	.....	.....
4 and 11	1.0	2.5	4.5	0.582	.0302	.....	.....
5 and 12	50.0	None	4.5	1.778	.0178	3.191	.0619
6 and 13	50.0	1.0	4.5	1.362	.0261	2.351	.1290
7 and 14	50.0	2.5	4.5	1.275	.0320	1.851	.1416

* Amount of roots inadequate for analysis in the low phosphate series.

Effects of aluminum in culture solutions upon the phosphate concentration of the cell sap

In order to study the nature of the interaction of phosphorus and aluminum within the plant, samples of the clear exuded sap of the lettuce plants were collected and analyzed for inorganic phosphorus. The method of obtaining this sap was essentially the same as that described in a previous paper (16), the sap being collected in a pipette attached by a rubber tubing to the cut end of the stem near the roots. The time of cutting the stems and the period of sap collection were the same for each group of plants. These are given in more detail in the notes at the bottom of tables 7 and 8. As previously explained the lettuce plants had been supplied with aluminum- and phosphate-containing solutions on alternate days.

The results of the first experiment with the plant sap are given in table 7. If the low-phosphate series plants are first considered it will be noted that the plants in the phosphate solutions contained about 200 p.p.m. of PO_4 in their sap. The plants that were in the "minus-PO_4" cultures, on the other hand, contained only small amounts of phosphate, and the plants which had received aluminum had a much lower concentration of phosphate in their sap than those that did not receive aluminum. Since the number of plants studied is so small, no significance can be attached to relatively small differences in the average PO_4 concentration of the sap samples obtained while the plants were in phosphate solution. Some later data obtained with corn indicate that if a large number of plants had been studied, small but significant differences might

TABLE 7

Phosphate concentration in the sap of lettuce plants as affected by the aluminum in the culture solution

ALUMINUM TREATMENT GIVEN PLANTS WHEN IN "MINUS-PO_4" SOLUTION	PLANTS IN "PO_4" SOLUTION DURING PERIOD OF COLLECTING SAP			PLANTS IN "MINUS PO_4" SOLUTION DURING PERIOD OF SAP COLLECTION		
	Plant[*] number	PO_4 in sap	Average PO_4 in sap	Plant number	PO_4 in sap	Average PO_4 in sap
		p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*
Low Phosphate Series (1.0 p.p.m. PO_4)[†]						
None (pH 6.0).....	8B − 1	253.0	253.0	8D − 1	77.0	77.0
None............	2A − 1	165.0	195.2	2C − 1	21.3	22.8
	9B − 1	225.5		9D − 2	24.2	
1.0 p.p.m.........	3A − 2	214.5	220.0	3C − 1	13.5	13.5
	10B − 1	225.5		10D − 1	13.5	
High Phosphate Series (50 p.p.m. PO_4)[‡]						
None	5A − 1	317.4	422.2	5C − 1	396.0	385.8
	12B − 1	448.3		5C − 2	387.5	
	12B − 2	501.0		12D − 1	374.0	
1.0 p.p.m.........	6A − 1	379.2	496.4	6C − 1	96.3	79.5
	13B − 1	458.1		6C − 2	62.6	
	13B − 2	651.8				
2.5 p.p.m.........	7A − 1	389.0	317.6	7C − 1	41.5	53.8
	14B − 1	283.3		7C − 2	66.0	
	14B − 2	280.5				

[*] The first two figures in each case refer to the culture number and the third to the plant number.

[†] The plants were cut at 10 a.m. and the sap was collected during a period of 31 hours. The plants had been in the same solution in which they were during the period of sap collection for a period of 17 hours previous to cutting the plants.

[‡] The plants were cut at 10 a.m. immediately after being placed in the desired solutions. The values given for the PO_4 concentration of the sap represent averages of samples removed after 8 hours and of samples removed after an additional 16 hours.

also have been noted in favor of the plants which had not previously been treated with aluminum. The results with the plants of the high-phosphate series are much the same. As would be expected, however, the concentration of the phosphate in the sap is considerably higher. The plants which were in the "minus-PO_4" solutions during the period of sap collection and which received aluminum had only one-seventh to one-fifth as high a concentration of phosphate in their sap as similar plants which did not receive aluminum.

In the second experiment, the results of which are given in table 8, the plants after being cut were placed in the "no-phosphate" solutions for a period of 19 hours, after which time they were transferred to a complete nutrient solution containing 50 p.p.m. PO_4. Sap samples were removed after the first 19 hours and again after additional periods of 6 and 20 hours. As will be noted, the results obtained substantiate those given in table 7. The plants which received 1.0 p.p.m. of aluminum and no phosphate during the period of collecting the sap contained only 82 p.p.m. of PO_4, whereas the plants which received no aluminum and no phosphate contained an average of 289 p.p.m. of PO_4 in their sap. After the plants were transferred to the phosphate solutions, the concentration of phosphates in the sap of all the plants rapidly increased, and after 26 hours the plants which had previously been in aluminum contained as high a concentration of phosphorus as those that had not.

TABLE 8

Phosphate concentration of the sap of lettuce plants when alternated from "no-PO_4" solutions some of which contained aluminum to phosphate cultures containing 50 p.p.m. of PO_4

ALUMINUM IN "NO-PHOSPHATE" CULTURES	PLANT NUMBERS	PHOSPHATE CONCENTRATION OF SAP					
		0–19 hours*		19–25 hours		25–45 hours	
		Single	Average	Single	Average	Single	Average
p.p.m.							
None	5A – 2	319	289	451	407	495	437
None	12D – 2	259		363		379	
1.0	6A – 2	72	82	231	270	421	467
1.0	13D – 2	91		308		512	

* Refers to number of hours after plants were cut for collection of sap. During the first 19 hours the plants were in the "no-PO_4" cultures after which time they were in culture solutions containing 50 p.p.m. of PO_4. Previous to cutting, the plants had been in culture solutions containing 50 p.p.m. of PO_4.

These studies of the plant sap establish the fact that the presence of aluminum in the culture solution may cause injury by reducing the concentration of soluble phosphate in the plant sap. Although the exact nature of this action is not definitely established, it would appear that in the presence of aluminum in the soil or culture solution the aluminum absorbed precipitates some phosphate within the plant and thus decreases the translocation of phosphate to the growing aerial parts. This is undoubtedly a reason for the fact that plants receiving large amounts of phosphate are less injured by aluminum than those receiving only small amounts of phosphate. In the former case enough phosphate is available, in addition to that which is precipitated within the plant by aluminum to produce good plant growth.

It does not necessarily follow, of course, that, as a result of this precipitating action of aluminum on phosphate within the plant, the plants receiving high amounts of aluminum will contain a higher percentage of phosphate than those which receive no aluminum. It will be noted in table 7 that the reverse is true. The presence of aluminum decreased the percentage of phosphate within the plant. The probable reason for this is that the injury from aluminum to the root hairs and root system is not materially overcome by the large additions of phosphate. Therefore, the injured root system is able to absorb phosphate less readily, and the plants will consequently contain less phosphate than those which did not receive aluminum, even though the amount of phosphate absorbed by the former may be high enough to produce good growth of top.

CONCLUSIONS

The results obtained in this investigation definitely show that the problem of aluminum toxicity and phosphate availability are very closely inter-related. The so-called toxic action of soluble aluminum in very acid soils can be ascribed to two general causes. First, aluminum in high enough concentrations directly injures the root hairs, and thus causes a stunted and poor development of the roots. As a result, plants are retarded in their development partly because of the direct injury and partly because of the greater difficulty in obtaining moisture and nutrient elements from the soil or culture solutions (11). The addition of large amounts of phosphate to soils functions in this respect not only in precipitating some aluminum from the soil solution but also indirectly by enabling the restricted root system to obtain a sufficient amount of available phosphate for nutritional purpose. The large amounts of phosphate, however, reduce the direct injury to the roots only inasmuch as aluminum is precipitated out of solution. In the culture solution experiment with lettuce, the high concentration of phosphate did not overcome the direct injury of aluminum to the roots, and the yield of top, although much better with the high than with the low amounts of phosphate, was definitely lower than in the absence of aluminum. Secondly, the aluminum absorbed by the plant precipitates part of the phosphate present and thus interferes with phosphate translocation and assimilation. In these experiments it was definitely shown that the concentration of inorganic phosphate in the cell sap of lettuce was greatly reduced by the presence of aluminum in the culture solution. Naturally, the injury due to this cause will be greater the lower the amounts of available soil phsophorus. The addition of large amounts of phosphate apparently supplies the plant with enough phosphorus to precipitate the aluminum absorbed and in addition to meet the phosphate needs for nutritional purposes.

Under field conditions it may be expected that less injury will be obtained from soluble aluminum in soils which are well supplied with available phosphate than in those which are not. This does not necessarily mean a high concentration of phosphate in the soil solution, but a supply of phosphate which is readily replenished as the growing plant removes that which is present in the soil solution.

SUMMARY

The beneficial action of large amounts of phosphate in reducing aluminum toxicity was studied with both soil and solution cultures. In the soil culture investigations various kinds and amounts of phosphate compounds were added to a very acid soil, and the effect on plant growth and on the pH and the aluminum, phosphorus, and calcium concentrations of the displaced soil solution was studied.

Monosodium and monocalcium phosphate additions reduced the aluminum concentration in the soil solution as a result of both the increased pH and the precipitation of aluminum phosphate.

Superphosphate even in high amounts (2,000 pounds P_2O_5 per acre) did not affect the pH of the soil but reduced the concentration of aluminum in the soil solution from about 18 to 10 p.p.m.

Good growth of barley and of alfalfa was obtained in the presence of 8 to 10 p.p.m. of aluminum where large amounts of superphosphate had been added. This is taken to indicate that the beneficial action of large phosphate additions to acid soils is partly due to a reaction of aluminum and phosphate within the plant and not entirely to the precipitation of aluminum in the soil solution.

Cos lettuce was grown in solution cultures receiving two different concentrations of phosphate and various concentrations of aluminum. The plants were alternated daily between phosphate- and aluminum-containing solutions in order to prevent the precipitation of aluminum as the phosphate. It was found that in the high-phosphate series (50 p.p.m. PO_4) the yields were reduced only 23 per cent from the presence of 2.5 p.p.m. aluminum, whereas in the low-phosphate series (1.0 p.p.m. PO_4) the reduction in yield was 71 per cent. Moreover, the average percentage of aluminum was as high in the plants which received high amounts of phosphate as in those which received the small amounts.

Samples of plant sap of the lettuce were collected and analyzed for inorganic phosphate. It was found that the phosphate concentration of the sap of the plants receiving aluminum was in some cases only one-fifth to one-seventh as high as from those which did not receive aluminum. This is considered particularly significant since it indicates that aluminum precipitates phosphate within the plant. When the plants were placed back in solutions containing high amounts of phosphorus, the phosphate concentration of the sap of all plants increased rapidly.

The results of these studies are believed to establish the fact that large phosphate additions to acid soils reduce the injurious action of aluminum not only by tending to precipitate it from the soil solution, but also by precipitating aluminum within the plant and by supplying sufficient phosphate for plant growth. In the presence of small amounts of available phosphate, injury to the roots is greater, first, because of the presence of more aluminum in the soil solution and, secondly, because the plants suffer more from a lack of phosphate as a result of the lessened absorption and of the precipitation of a greater proportion of the phosphate within the plant by aluminum.

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PLATE 1

EFFECT OF DIFFERENT PHOSPHATE CONCENTRATIONS ON THE INJURY TO COS LETTUCE FROM SOLUBLE ALUMINUM

FIG. 1. Low-Phosphate Series (1 p.p.m. PO_4)

CULTURE NUMBER	pH	p.p.m. Al
1C	6.0	0
2C	4.5	0
3C	4.5	1.0
4C	4.5	2.5

FIG. 2. High-Phosphate Series (50 p.p.m. PO_4)

CULTURE NUMBER	pH	p.p.m. Al
1B*	6.0	0
12B	4.5	0
13B	4.5	1.0
14B	4.5	2.5

* Received only 1.0 p.p.m. PO_4.

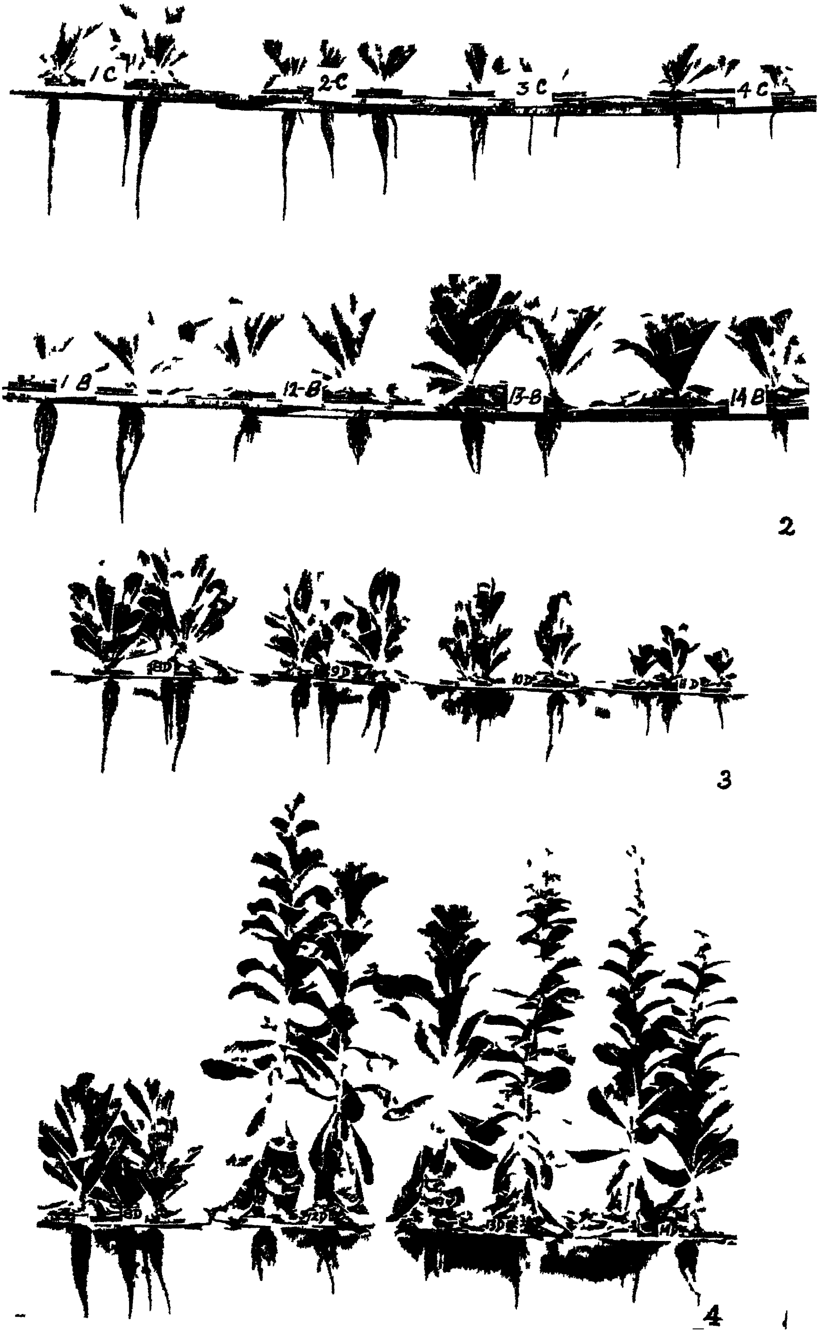
FIG. 3. Low-Phosphate Series (1 p.p.m.)

CULTURE NUMBER	pH	p.p.m. Al
8D	6.0	0
9D	4.5	0
10D	4.5	1.0
11D	4.5	2.5

FIG. 4. High-Phosphate Series (50 p.p.m. PO_4)

CULTURE NUMBER	pH	p.p.m. Al
8D*	6.0	0
12D	4.5	0
13D	4.5	1.0
14D	4.5	2.5

* Received only 1.0 p.p.m. PO_4.



Figs 1–4

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: XII. THE AMPHOTERIC NATURE OF SOILS IN RELATION TO ALUMINUM TOXICITY[1]

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The utilization of relatively large quantities of physiologically acid fertilizers and the extensive research in soil science in recent years have directed considerable attention to the study of soluble aluminum in connection with plant injury in acid soils. Distinct differences of opinion have been expressed with reference to the rôle of soluble aluminum salts in toxicity to plant growth. The purpose of the present study was to determine the correlation between the injury to plant seedlings and the amphoteric properties of distinctly different acid soil types.

The question of aluminum toxicity in soils has been so completely reviewed by Mirasol (16), Magistad (13), McGeorge (11, 12), and others (2, 3, 15, 17, 20, 23) that it seems hardly necessary to include a review of the literature here. However, it is of interest to know that aluminum exists to some extent in most plants and that in many cases its percentage is as great as that of iron. Also, from the data accumulated, the conclusion is drawn that aluminum is toxic to plant growth when present in solution in sufficient quantities. Furthermore, several papers have appeared showing that aluminum toxicity appears at one pH value in one soil and at another pH value, higher or lower, in another soil. Then the work of Pierre (18) cannot be overlooked in which he found a rather good correlation between the percentage base saturation and plant injury on acid soils. It is believed that the data and explanation presented in this paper are pertinent to these facts.

Because of the amphoteric nature of the soil complex and because of the fact that the isoelectric point differs greatly in different types of soils, the pH at which Al and other of the basoid constituents begin to ionize and become soluble may be expected to vary accordingly.

A high isoelectric point means a relatively active basoid group in which the Al becomes ionized at comparatively high pH values. The basic residue is greater, that is, the degree of combination with the acidoid group is smaller, and the Al is therefore more easily liberated by hydrolytic cleavage, resulting in solution of the ionized group.

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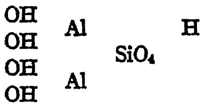
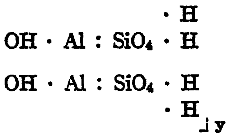
A low isoelectric point means on the other hand a smaller and less active basic residue and therefore a more firmly associated aluminum which becomes ionized and soluble only at comparatively low pH.

The following formulas might serve to illustrate the relationship:



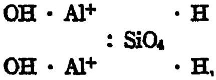
$$\begin{array}{llll} \mathrm{OH} & & & \\ \mathrm{OH} & \mathrm{Al} & & \mathrm{H} \\ & & \mathrm{SiO_4} & \\ \mathrm{OH} & \mathrm{Al} & & \\ \mathrm{OH} & & & \end{array} \qquad (A)$$



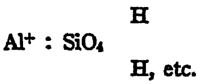
$$\left.\begin{array}{r} \cdot\,\mathrm{H} \\ \mathrm{OH}\cdot\mathrm{Al} : \mathrm{SiO_4}\cdot\mathrm{H} \\ \mathrm{OH}\cdot\mathrm{Al} : \mathrm{SiO_4}\cdot\mathrm{H} \\ \cdot\,\mathrm{H} \end{array}\right]_y \qquad (B)$$

The complex represented by formula (*A*) has a ratio of $SiO_2/Al_2O_3 = 1$ and would be isoelectric at a high pH, whereas the complex represented by formula (*B*) has a ratio of $SiO_2/Al_2O_3 = 2$ and would be isoelectric at a lower pH. Since the positive charge developed on the acid side of the isoelectric point is due to the ionization of the basic group it is obvious that Al ionizes in complex (*A*) at a higher pH than that at which it ionizes in complex (*B*). Also, since the basic residue is greater in (*A*) than in (*B*) it is clear that, at a given low pH, the ionization of the basic group will be greatest in (*A*) as will also its dispersion and solution in the form of single ions.

From the fact that Al and Fe as cations carry silica to the cathode compartment in the electrodialysis of soils (14, XI) we may assume that the cations which at first pass into solution are of a complex nature such as



$$\begin{array}{lcr} \mathrm{OH}\cdot\mathrm{Al^+} & & \cdot\,\mathrm{H} \\ & :\mathrm{SiO_4} & \\ \mathrm{OH}\cdot\mathrm{Al^+} & & \cdot\,\mathrm{H,} \end{array}$$



$$\begin{array}{ll} & \mathrm{H} \\ \mathrm{Al^+} : \mathrm{SiO_4} & \\ & \mathrm{H,\ etc.} \end{array}$$

Complete hydrolysis with the formation of Al^{+++} ions can only take place at very low pH.

SOILS USED

In order to determine the relationship of the amphoteric nature of the soil complex to the pH at which toxic effects on plant growth become manifest, three soils representing the extreme as well as the intermediate type of amphoterism were selected namely a Nipe laterite from Cuba, a Sassafras loam from New Jersey and a Sharkey clay from Mississippi. The amphoteric properties of these soils have been discussed in the preceding articles of this series (14, VI, X). Their combining capacities with cations and anions and their pH of exchange neutrality are shown in figure 28 (14, X). The latter values in a Na_2SO_4 solution were as follows: Nipe, 6.2, Sassafras, 4.6; Sharkey, 3.7.

The isoelectric points of the Nipe and the Sassafras soil colloids in NH_4Cl solution were found at somewhat lower values, or at pH 6.0 and 4.4 respectively (14, VI). For the Sharkey soil no cataphoretic isoelectric point has been found. This apparently applies to all soil colloids having a high silica/sesquioxide ratio. Yet these materials react amphoterically, that is, they combine with anions as well as with cations.

The reason why these colloids do not become positively charged must be ascribed to their low isoelectric point. At low pH values the basic groups (i.e., Al and Fe) not only ionize but are more extensively liberated by hydrolysis from their combination with the acidic groups. This results in the solution and separation from the colloidal complex of the positive ions which otherwise would have charged the colloid electropositively. Thus the Sharkey colloid was found not to adsorb more than mere traces of Cl ions from a chloride solution at low pH. But considerable Al and Fe were found in solution (14, VI). The colloid did obviously react with (adsorb) the anions but the products of the reaction (adsorption) were soluble. It might be said that the diffusible, "adsorbed ion" pulled the originally non-diffusible, "adsorbing ion" out into the solution. Although this will prevent a change of charge of the colloid, it will not affect the point of exchange neutrality, since this depends only upon the number of H and OH ions removed from the solution independent of whether or not the products of the reaction are soluble or insoluble. The latter point is therefore a more reliable indication of the amphoteric nature of a colloid than is the cataphoretic isoelectric point.

TECHNIQUE OF TESTING INJURY

Wheat seedlings were used as the test crop. After the seedlings had sprouted in moist sand and grown to about 1 cm. in height, they were transplanted to the soils. The moisture content was held at 40 per cent of the water retaining power of the soil. A preliminary experiment showed this to be a desirable moisture content. The percentage of moisture was maintained by weighing the cultures daily or as often as necessary. The tests were made in small sample bottles containing 100 gm. of soil. The pH values of the samples were adjusted by the addition of HCl and $Ca(OH)_2$ to the electrodialyzed soils. The acid and the base were added to a 1:2 ratio of soil to water and the mixture was allowed to stand over night. The excess water was then evaporated in a warm place, an electric fan being used to hasten the evaporation.

The water-soluble constituents were determined by submerging the moist soil, after the plants were removed, in a 1:1 ratio of soil to water, allowing it to stand over night and then placing it on a filter and washing it with small portions of water until a volume of 200 cc. was obtained. The aluminum and iron, or R_2O_3, were determined in the total volume. Dialysis of the soil in collodion bags was used to test the validity of the method. The results were found to check very well if the soils were dialyzed for 5 days as recommended by Magistad (13). Iron and aluminum separations were made in the first series, but since iron occurred only in very small quantities, no further separations were made. The pH values were determined colorimetrically.

TESTS WITHOUT NUTRIENTS

The first experiments to be considered are those in which no nutrients were added. Two series were made from each soil; one in June extending over a period of 12 days and another in September for a period of 8 days. The results are shown in tables 101 to 103.

TABLE 101

Development of wheat seedlings in the electrodialyzed Sharkey soil at varying pH values

NUMBER	GROWTH MADE IN 12 DAYS	APPARENT INJURY	GROWTH	0.1 N HCl ADDED	PER CENT OF SATURATION CAPACITY WITH CALCIUM	pH AT BEGINNING OF EXPERIMENT	pH AT END OF EXPERIMENT	R_2O_3*	DRY WEIGHT GROWTH†
			cm.	*cc.*				*mgm.*	*mgm.*
				First series—June, 1931					
1	Dead	Yes	0	20		2.6	2.6	52.8	0.0
2	None	Yes	2	10		2.8	2.8	29.0	11.1
3	Some	Yes	3	5		3.0	3.2	16.4	21.2
4	Some	Yes	5	0		3.4	3.4	1.6	26.6
5	Good	No	7		10	3.5	3.6	Trace	41.2
6	Good	No	7		20	3.9	4.0	Trace	39.2
7	Good	No	7		40	4.6	4.6	Trace	39.0
				Second series—September, 1931					
	8 DAYS								
1	Dead	Yes	0	20			2.8	41.2	0.0
2	Dead	Yes	0	10			3.0	31.8	0.0
3	Dead	Yes	0	5			3.2	22.8	5.5
4	Alive	Yes	2	0			3.5	3 5	11.3
5	Good	No	5		10		4.0	Trace	20.6
6	Good	No	6		20		4.4	Trace	25.5

* Water-soluble R_2O_3 in milligrams per 100 gm. of soil, principally Al_2O_3; Fe_2O_3 and P_2O_5 occurred as trace only in Nos. 1, 2, and 3.

† Dry weight of the growth of the blades in milligrams.

The Sharkey soil. It will be noted that the last pH of good growth in this soil, under these conditions, is at about 3.6. All plant characteristics indicated this point as the lowest pH value of good growth. Below this pH, Al (R_2O_3) began to pass into solution in increasing quantities. The second series agrees in a general way with the first in spite of the seasonal variation.

The Sassafras soil. The results from the Sassafras soil show a very interesting comparison in respect to the injury point. Although the lowest pH of good growth in the Sharkey series was at 3.6, that for the Sassafras soil wasa t pH 4.4. This is in agreement with the Al solubility, which begins at a higher pH in the Sassafras soil. At pH 3.6 this soil yielded considerable quantities of soluble aluminum whereas the Sharkey yielded a mere trace of this element. This is all in agreement with the amphoteric nature of the colloidal complex in the two soils.

TABLE 102

Development of wheat seedlings in the electrodialyzed Sassafras soil at varying pH values

ORDER OF SERIES	GROWTH MADE IN 12 DAYS	APPARENT INJURY	GROWTH	0.1 *N* HCl ADDED	PER CENT OF SATURATION CAPACITY WITH CALCIUM	pH AT BEGINNING OF EXPERIMENT	pH AT END OF EXPERIMENT	R_2O_3*	DRY WEIGHT GROWTH†
			cm.	*cc.*				*mgm.*	*mgm.*
				First series—June, 1931					
1	Dead	Yes	0	20		2.9	3.0	32.0	14.5
2	Some	Yes	3	10		3.3	3.4	9.6	16.1
3	Fair	Yes	5	5		3.6	3.6	5.2	21.0
4	Good	No	9	0		4.4	4.4	0.4	35.6
5	Good	No	9		5	4.5	4.5	Trace	42.2
6	Good	No	10		10	4.7	4.8	Trace	47.7
7	Good	No	8		20	4.9	4.9	Trace	38.2
				Second series—September, 1931					
	8 DAYS								
1	Dead	Yes	0	40			2.9	95.4	0.0
2	Dead	Yes	0	30			3.1	60.1	0.0
3	Dead	Yes	0	20			3.2	34.8	0.0
4	Dead	Yes	0	10			3.5	18.6	0.0
5	Alive	Yes	1	5			3.8	5.2	6.5
6	Good	No	10	0			4.6	2.0	40.5
7	Good	No	11		5		4.7	Trace	44.5
8	Good	No	11		10		5.2	Trace	42.8

* Water-soluble R_2O_3 in milligrams per 100 gm. of soil, principally Al_2O_3; Fe_2O_3 and P_2O_5 occurred as trace only in Nos. 1, 2 and 3.

† Dry weight of the growth of the blades in milligrams.

The Nipe soil. The last point at which good growth occurred in this soil was at about pH 4.8. The injury must, however, be ascribed to manganese, because at the highest pH at which injury was evident there was no Al in solution, whereas appreciable quantities of Mn were found. Manganese injury in laterite soils has been reported by McGeorge (11, 12) and others. The manganese instead of the aluminum content in the soil extract is therefore recorded in the table. At lower pH the injury is undoubtedly due to the combined effect of Al and Mn. It appears that Mn ionizes and becomes soluble at a somewhat higher pH than Al. The Sharkey and Sassafras soils contain very small amounts of Mn whereas the Nipe laterite contains 0.74 per cent MnO. This laterite contains 62.51 per cent Fe_2O_3 and only 15.84 per cent Al_2O_3. Aluminum toxicity would therefore not be expected at as high a pH as would be the case if the proportion of Al were higher. As a member in a series for the study of Al toxicity this soil is not so well suited but it nevertheless occupies its theoretical position in the order of toxicity as related to the amphoteric nature of the soils.

TABLE 103

Development of wheat seedlings in the electrodialyzed Nipe soil at varying pH values

NUMBER	GROWTH MADE IN 12 DAYS	APPARENT INJURY	GROWTH	0.1 *N* HCl ADDED	PER CENT OF SATURATION CAPACITY WITH CALCIUM	pH AT END OF EXPERIMENT	Mn_2O_3*	DRY WEIGHT GROWTH†
			cm.	*cc.*			*mgm.*	*mgm.*
				First series—June, 1931				
1	Alive	Yes	3	20		4.2	6.6	9.0
2	Some	Slight	6	10		4.6	3.4	34.6
3	Good	No	7	5		4.8	1.1	41.1
4	Good	No	7	0		5.6	Trace	49.2
5	Good	No	7		5	5.9	Trace	41.2
6	Good	No	7		10	6.2	None	39.1
7	Good	No	6		20	6.4	None	32.0
				Second series—September, 1931				
	8 DAYS							
1	Dead	Yes	0	30		4.2	7.6	8.5
2	Alive	Yes	6	20		4.3	6.2	20.5
3	Good	Slight	12	15		4.5	3.6	48.8
4	Good	No	14	10		4.7	1.0	49.8
5	Good	No	14	5		5.2	0.5	48.0
6	Good	No	14	0		5.6	0.2	46.0

* Water-soluble Mn_2O_3 in milligrams per 100 gm. of soil.

† Dry weight of the growth of the blades in milligrams.

Another result brought out in the tables and related to the amphoteric nature of the three soils should be pointed out. It will be noted that the Sharkey soil is toxic in its electrodialyzed, i.e., unsaturated, condition and permits good growth only when saturated to the extent of 10 per cent of its cation combining capacity (at pH 7.0) with $Ca(OH)_2$. The Sassafras soil permits good growth in its unsaturated condition but is toxic with 5 cc. 0.1 *N* HCl. The Nipe soil permits good growth with 5 or even 10 cc. 0.1 *N* HCl and becomes toxic only with greater quantities of acid. Thus we see that, whereas the pH injurious to plants is highest in the case of the Nipe, its ultimate pH and its combining capacity for acids are at the same time so much higher that the injury to plants (from Al or Mn toxicity) must always be more remote in this soil than in a soil of the Sharkey type at the same degree of saturation. It appears, indeed, doubtful that a lateritic soil should ever become toxic except under the most abnormal conditions.

According to the aforementioned results, the Sharkey soil can become toxic to plants merely by its own unsaturation whereas the Sassafras and especially the Nipe soil could not be toxic without the introduction of free acids beyond the point of complete unsaturation. The presence of soluble salts (nutrients) greatly modifies the aforementioned results, as will be shown later. Before presenting the results obtained when nutrient salts were added to the soils we shall discuss the effects of increasing the acidoid and basoid constituents of the soil complex, that is to say, the effects of lowering and raising the isoelectric point of the complex.

TABLE 104

Development of wheat seedlings in an aluminated and electrodialyzed Sharkey soil at various pH values

NUMBER	GROWTH MADE IN 12 DAYS	APPARENT INJURY	GROWTH	0.1 *N* HCl ADDED	PER CENT OF SATURATION CAPACITY WITH CALCIUM	pH AT BEGINNING OF EXPERIMENT	pH AT END OF EXPERIMENT	R_2O_3*	DRY WEIGHT GROWTH†
			cm.	*cc.*				*mgm.*	*mgm.*
1	Alive	Yes	4	40		3.9	4.1	18.2	12.9
2	Alive	Yes	5	20		4.2	4.3	16.4	14.6
3	Alive	Yes	6	10		4.4	4.5	9.2	15.4
4	Good	No	13	5		4.7	4.8	4.4	49.1
5	Good	No	14	0		5.8	5.6	0.4	69.2
6	Good	No	13		2.8	6.2	6.0	0.0	50.0
7	Good	No	13		5.5	6.6	6.3	0.0	59.8
8	Good	No	13		11.3	6.8	6.7	0.0	69.9

* Water-soluble R_2O_3 in milligrams per 100 gm. of soil, principally Al_2O_3.

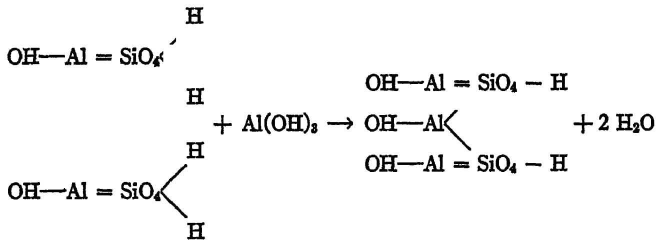
† Dry weight of the growth of the blades in milligrams.

EFFECTS OF ALUMINUM HYDROXIDE

The isoelectric point of the soil complex may be raised to any value by precipitating the soil suspension with aluminum as described in a previous paper (14, IV). A suspension of the Na-saturated Sharkey soil was precipitated with $AlCl_3$ in the proportion of 1.0 millimol to each gram of soil. The precipitation was done at pH 6.0 by the addition of NaOH. The soil was then dried and electrodialyzed free from diffusible ions. The pH of exchange neutrality (in *N* Na_2SO_4 solution) was now 5.0 as compared to 3.7 of the original soil. The development of the wheat seedlings in this soil is shown in table 104.

We note that the injury point in the aluminated soil has been raised to between the pH values 4.5 and 4.8 as compared to 3.6 in the original soil. It should also be noted that whereas the original soil required a 10 per cent saturation with Ca $(OH)_2$ to reduce the Al concentration below its critical (toxic) value, the aluminated soil permits a good growth not only in its unsaturated condition but, like the Nipe laterite, after the addition of 5 cc. 0.1 *N* HCl as well. The isoelectric point and the ultimate pH of the aluminated Sharkey are so much higher that very little Al passes into solution from the completely unsaturated soil complex. But because of the increase in the quantity and activity of the basoid group in the synthetic complex the ionization and solution of Al are much greater at a given low pH in the latter than in the original soil.

The activation of the basoid group and the inactivation of the acidoid group by $Al(OH)_3$ might be illustrated as follows:



This accounts for the raising of the isoelectric point, the ultimate pH, and the pH of exchange neutrality and for the lowering of the cation exchange capacity (at pH 7.0).

EFFECTS OF SILICIC ACID

The isoelectric point, the ultimate pH, and the pH of exchange neutrality may be lowered and the cation exchange capacity (at pH 7.0) may be increased by increasing the acidoid content of the soil complex. Theoretically this should also lower the point of Al toxicity. To test this a suspension of the Sassafras soil was precipitated together with a solution of Na silicate by adjusting the pH to a value of 3.2 by the addition of HCl. The soil and the silicate were mixed in the proportion of 1 gm. soil to 7.5 millimol SiO_2. At a pH of 3.2 the soil complex was electropositive and reacted as a base with some of the silicic acid. The supernatant liquid was filtered off and the soil was dried and then electrodialyzed free from diffusible ions.

The pH of exchange neutrality of the silicated soil was 4.1 as compared to 4.6 in the case of the original soil. The development of the wheat seedlings in this soil is shown in table 105.

Although the pH values are too far apart for an accurate determination of the point of injury it is very evident from a comparison of tables 102 and 105 that silicic acid lowered this point. The silicated Sassafras soil permitted good growth and yielded very little soluble Al at pH 4.0.

At lower pH values the silicated soil yielded, for some reason, much more soluble Al than did the original soil. The freshly precipitated silica was undoubted more soluble than the silica of the original soil and it is possible that the soluble silicic acid, by association with the Al ions, rendered the latter more stable in solution. Without any silica in solution the Al ions could only associate with the anions of the colloidal complex and would then not appear as soluble Al to the same extent.

It should also be noted that the plants were alive in the silicated soil at a pH of 3.1, at which 67.4 mgm. R_2O_3 went into solution. In the original soil (table 102) the plants were dead when the R_2O_3 content reached 32.0 mgm. in the first series and 18.6 mgm. in the second series. It is possible that the Al ion is less toxic (less diffusible through the cell membranes?) in association with the silicate ion than in the free condition. This might account for the fact that Al appears to be more toxic in water cultures.

TABLE 105

Development of wheat seedlings in a silicated and electrodialyzed sassafras soil at various pH values

NUMBER	GROWTH MADE IN 12 DAYS	APPARENT INJURY	GROWTH	0.1 *N* HCl ADDED	PER CENT OF SATURATION CAPACITY WITH CALCIUM	pH AT BEGINNING OF EXPERIMENT	pH AT END OF EXPERIMENT	R_2O_3*	DRY WEIGHT GROWTH†
			cm.	*cc.*				*mgm.*	*mgm.*
1	Dead	Yes	0	40		1.8	2.6	117.2	9.2
2	Alive	Yes	4	25		2.8	3.1	67.4	10.0
3	Alive	Yes	5	10		3.0	3.4	43.6	11.0
4	Good	No	12	0		3.7	4.0	0.8	51.8
5	Good	No	13		2.8		4.3	0.4	60.8
6	Good	No	13		6.9		4.6	0 4	60.0
7	Good	No	10		13 8		5.5	Trace	50.2

* Water-soluble R_2O_3 in milligrams per 100 gm. of soil, principally Al_2O_3.

† Dry weight of the growth of the blades in milligrams.

EFFECT OF HUMIC ACIDS

The Nipe soil was treated with 2.5 per cent of electrodialyzed humic acid. The development of the seedlings in the humated soil is shown in table 106.

By comparing tables 103 and 106 we note that even as little as 2.5 per cent of humic acid caused a distinct lowering of the injury point, which in the humated soil is below pH 4.4, whereas a pH of 4.6 proved injurious in the original soil. The solubility of the manganese was also suppressed by the humic acid. The same is true of Al, as shown by Burgess and Pember (5).

The suppression of the activity of the basoid groups, by the addition of silicic and humic acids, is undoubtedly due to a combination between the basic

TABLE 106

Development of wheat seedlings in a humated and electrodialyzed Nipe soil at various pH values

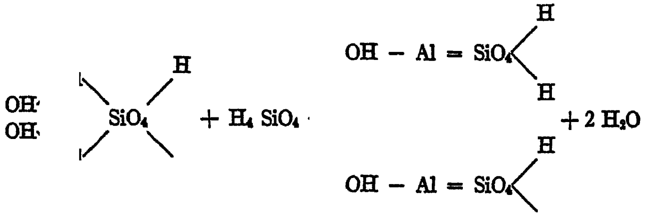
November, 1931

NUMBER	GROWTH MADE IN 12 DAYS	APPARENT INJURY	GROWTH	0.1 *N* HCl ADDED	PER CENT OF SATURATION CAPACITY WITH CALCIUM	pH AT BEGINNING OF EXPERIMENT	pH AT END OF EXPERIMENT	Mn_2O_3*	DRY WEIGHT GROWTH†
			cm.	*cc.*				*mgm.*	*mgm.*
1	Poor	Yes	7	20		4.0	4.2	3.2	36.0
2	Good	No	12	10		4.2	4.4	1.4	55.0
3	Good	No	13	5		4.8	5.3	0.8	72.8
4	Good	No	12	0		5.8	5 6	0.4	70.1
5	Good	No	13		5	6.0	5 8	Trace	88.2

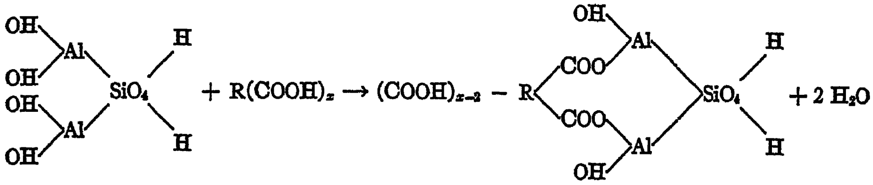
* Water-soluble Mn_2O_3 in milligrams per 100 gm. of soil.

† Dry weight of the growth of the blades in milligrams.

residue at the surface of the particles and the added acids. We might illustrate our conception of this reaction with silicic acid as follows:



and with humic acid, for which we write $R(COOH)_x$, as follows:



This accounts for the lowering of the isoelectric point, the ultimate pH, and the pH of exchange neutrality as well as for the increase in the cation exchange capacity (at pH 7.0). Whereas the common anions, which form soluble salts with Al and Mn, increase the ionization and solubility of these elements, other anions (silicate, humate, phosphate, etc.) which form insoluble salts with these elements, suppress their ionization and solubility.

The phosphate ion lowered the isoelectric point and the point of injury as in the case of the silicic acid and humus. Because of a methodical error the results will, however, not be tabulated.

EFFECT OF NUTRIENT SALTS

A very interesting observation was the effect of adding nutrients to the electrodialyzed soils in the testing of the injury point to the seedlings. There is any number of possible proportions of nutrients to be added which would give a somewhat different injury point. The nutrients used were calculated from Shive's nutrient solution study (21). They were as follows: potassium sulfate, 0.1 millimol; calcium nitrate, 0.185 millimol; and magnesium chloride, 0.140 millimol per 100 gm. of soil. Phosphorus was avoided since it would materially change the complex. The seeds no doubt had enough phosphorus to complete successfully so short a growth period.

TABLE 107

Development of wheat seedlings in electrodialyzed Sharkey soil at various pH values in presence of nutrient salts

NUMBER	GROWTH MADE IN 12 DAYS	APPARENT INJURY	GROWTH	0.1 *N* HCl ADDED	PER CENT OF SATURATION CAPACITY WITH CALCIUM	pH AT BEGINNING OF EXPERIMENT	pH AT END OF EXPERIMENT	R_2O_3*	DRY WEIGHT GROWTH†
			cm.	*cc.*				*mgm.*	*mgm.*
				First series—June, 1931					
1	Dead	Yes	0	0		3.4	3.0	22.8	0.0
2	Some	Yes	5		10	3.6	3.2	8.1	54.0
3	Fair	Yes	7		20	4.0	3.6	7.3	64.2
4	Fair	Yes	7		30	4.7	4.1	6.0	67.2
5	Good	No	14		40	5.3	4.4	6.0	107.1
6	Good	No	12		50	5.6	4.6	5.0	80.4
7	Good	No	12		60	6.0	4.9	5.0	98.5
				Second series—September, 1931					
	8 DAYS								
1	Dead	Yes	0	10			2.9	20.3	4.5
2	Alive	Yes	2	0			3.1	8.5	5.0
3	Some	Yes	2.5		10		3.3	5.2	11.0
4	Some	Yes	3		20		3.7	3.3	18.6
5	Fair	Slight	5		30		4.0	3.8	22.0
6	Good	Good	7		40		4.3	3.0	30 8

* Water-soluble R_2O_3 in milligrams per 100 gm. of soil, principally Al_2O_3.

† Dry weight of the growth of the blades in milligrams.

It has been observed by Mattson that a mixture of salt and acid brings more aluminum into solution than the acid alone at the same pH. This being the case, the injury point with the soils would be expected to have been raised from the point found with the electrodialyzed soils. The antagonistic effect of the other ions would make the plants more resistant to injury from aluminum, however. It is noticed in the studies with the nutrients that the injury point was raised, i.e. the injury occurred at a higher pH value than when the straight electrodialyzed material was used alone. The plants made good growth at the following points in the series: Sharkey, pH 4.4; Sassafras, pH 4.6; and Nipe, pH 5.5, as compared to the following figures for the no nutrient series: Sharkey, pH 3.6; Sassafras, pH 4.4; and Nipe, pH 4.8. These results are shown in tables 107, 108, and 109.

The percentage saturation with $Ca(OH)_2$ at the point of injury has been raised from 10 to 40 per cent in the Sharkey, and from 0 to 10 per cent in the Sassafras, by the presence of the nutrient salts. The Nipe, which, without nutrients, permitted good growth with 10 cc. 0.1 *N* HCl added to the soil, is decidedly toxic where acid was added together with nutrient salts.

TABLE 108

Development of wheat seedlings in electrodialyzed Sassafras soil at various pH values in presence of nutrient salts

NUMBER	GROWTH MADE IN 12 DAYS	APPARENT INJURY	GROWTH	0.1 *N* HCl ADDED	PER CENT OF SATURATION CAPACITY WITH CALCIUM	pH AT BEGINNING OF EXPERIMENT	pH AT END OF EXPERIMENT	R_2O_3*	DRY WEIGHT GROWTH†
			cm.	*cc.*				*mgm.*	*mgm.*
				First series—June, 1931					
1	Alive	Yes	2	10		4.0	3.7	19.2	30.3
2	Fair	Yes	8	0		4.3	3.8	11.2	55.0
3	Good	Slight	10		5	4.8	4.5	4.4	70.0
4	Good	No	13		10	5.1	4.6	2.6	98.2
5	Good	No	11		15	5.4	5.1	2.2	68.2
6	Good	No	10		20	5.6	5.3	2.3	95.8
7	Good	No	10		30	5.8	5.4	2.2	82.0
				Second series—September, 1931					
	8 DAYS								
1	Alive	Yes	1	20			3.5	20.3	6.8
2	Some	Yes	2	10			3.8	7.4	18.0
3	Good	Slight	14	0			4.4	1.3	64.5
4	Good	Slight	15		5		4.7	1.1	85.8
5	Good	No	15		10		4.8	1.2	101.6
6	Good	No	16		15		5.0	0.8	81.6

* Water-soluble R_2O_3 in milligrams per 100 gm. of soil, principally Al_2O_3.

† Dry weight of the growth of the blades in milligrams.

The salts increased the solubility of Al and Mn. The acid anions displace in high concentrations more OH ions from the basic residue of the complex, thereby increasing the ionization and solubility. Just as the monovalent cations increase the ionization of the acid residue at high pH values so the monovalent anions increase the ionization of the basic residue at low pH as shown by cataphoresis (14, II). Another evidence in support of this explanation is the fact that chlorides inhibit the hydrolysis of $FeCl_3$ and, conversely, tend to redissolve the hydroxide at low pH values.

Another noteworthy fact is that the pH drift over the 12-day period in the Sharkey and Sassafras soils was downward whereas it had been slightly upward in the series with no nutrients. The displacement of H ions predominated in these soils. In the case of the Nipe the drift was upward below pH 5.5 and downward above this value in accordance with the high isoelectric point of this soil. The displacement of OH ions was here dominant at the lower pH values.

TABLE 109

Development of wheat seedlings in electrodialyzed Nipe soil at various pH values in presence of nutrient salts

NUMBER	GROWTH MADE IN 12 DAYS	APPARENT INJURY	GROWTH	0.1 *N* HCl ADDED	PER CENT OF SATURATION CAPACITY WITH CALCIUM	pH AT BEGINNING OF EXPERIMENT	pH AT END OF EXPERIMENT	Mn_2O_3*	DRY WEIGHT GROWTH†
			cm.	*cc.*				*mgm.*	*mgm.*
				First series—June, 1931					
1	Alive	Yes	2	30		4.0	4.3	6.4	22.3
2	Alive	Yes	4	20		4.3	5.0	1.2	80.1
3	Some	Yes	8	10		4.8	5.4	1.2	85.6
4	Good	No	15	0		5.4	5.5	0.8	170.6
5	Good	No	15		5	5.6	5.5	0.6	161.4
6	Good	No	16		10	5.9	5.6	0.4	163.1
7	Good	No	16		20	6.2	5.6	0.4	166.4
				Second series—September, 1931					
	8 DAYS								
1	Dead	Yes	0	60			3.9	6.2	6.2
2	Fair	Slight	12	30			4.4	0.9	74.8
3	Good	No	16	20			4.8	0.8	90.6
4	Good	No	16	10			5.0	0.6	74.2
5	Good	No	16	0			5.2	Trace	86.6

* Water-soluble Mn_2O_3 in milligrams per 100 gm. of soil.

† Dry weight of the growth of the blades in milligrams.

In most cases, especially in the Nipe soil, it was noted that the seedlings looked best at low pH values immediately above the point of injury. This might be due to the nutrient or stimulating effect of the same ion which in higher concentrations caused injury. Manganese in small quantities is now known to have a stimulating effect (4).

DISCUSSION

Data (5, 6, 18) have been presented to show that the injury to plants occurs at various pH values in different soils, and that active hydrogen is not the primary factor in producing this plant injury.

The fact that aluminum (2, 3, 7, 9, 10, 15, 22, 23) and manganese (4, 11, 12) are toxic to plant growth when present in sufficient quantities has been well established. Investigations in soil work (1, 3, 8, 14) show that toxicity has often occurred as a result of these elements going into solution.

It has also been found that organic matter counteracts the toxic effects of aluminum (5), and Pierre (18) has made the important observation that soils of relatively high percentage base saturation at low pH values yield, in general, less aluminum in solution than soils of low percentage base saturation.

This observation is restated in a very recent paper by Pierre, Pohlman, and McIlvaine (19), who add that the aluminum solubility is very materially affected by the concentration of soluble salts.

No satisfactory explanation has been given to account for all these facts and no explanation has, in our opinion, been possible without a recognition of the amphoteric nature of the soil complex.

The isoelectric point of the soil complex is governed by the relative activity of the acidic and basic residues. A low isoelectric point means a weak basic residue. The basic groups are then largely tied up in non-dissociable combinations with the acid groups within the complex. It requires, therefore, a low pH for the ionization and solution (by hydrolytic cleavage) of the basic groups, that is, Al, Fe, Mn, etc. A low isoelectric point means, on the other hand, a strong acid residue and a relatively high percentage saturation at a low pH.

A high isoelectric point means a relatively stronger basic residue. Ionization and solution of the basic groups take place at higher pH values. At a low pH, however, the percentage base saturation is low, which accounts for the observed (indirect) relationship between this value and aluminum solubility.

An introduction of additional acid groups (silicate, phosphate, humate, etc.) weakens the basic residue, lowers the isoelectric point, and renders the basic groups (Al, Fe, Mn, etc.) less ionizable and less soluble.

The addition of neutral salts has the opposite effect because their anions displace the OH ions of the basic residue to form dissociable compounds thus increasing the ionization and solution of the basic groups of the complex.

In conclusion it must be added that whereas the ionization of the *basic groups* of the soil complex at any pH must be definitely related to the isoelectric point it obviously does not follow that there must be the same definite relationship between the ionization and solubility of *aluminum* (or manganese or any one other element) and the isoelectric point. Aluminum makes up various proportions of the basic groups of the complex and will ionize only in proportion to its activity as compared to the activity of the other groups. Only in cases where aluminum is the only basic element present in the complex could we expect a definite relationship between aluminum solubility and the isoelectric point. It is not possible, therefore, to construct an exact curve relating aluminum solubility at various pH values to the isoelectric point or to the pH of exchange neutrality of soils. The relationship is further complicated by the presence of various proportions of the acid groups in the complex, such as silicate, humate, and phosphate, the compounds of which hydrolyze to different degrees. As far as relating Al toxicity to Al solubility is concerned the relationship is complicated by the solubility and toxic effects of Mn and possibly other elements.

SUMMARY

It has been shown that the pH at which sufficient aluminum becomes soluble to cause injury to plants is related to the amphoteric nature of the soil.

Three soils, a Sharkey clay, a Sassafras loam, and a Nipe laterite, representing a high, an intermediate, and a low silica/sesquioxide ratio in the colloidal fraction, were selected as media for the growth of wheat seedlings. The pH of exchange neutrality in N Na_2SO_4 solution was 3.7, 4.6, and 6.2 respectively whereas the lowest pH recorded for good growth in the electrodialyzed soils and in the absence of nutrient salts was 3.6, 4.4, and 4.8 respectively.

The higher the isoelectric point of a soil the higher is the point of injury. This is obviously because the activity, the ionization, and the solution of the basic groups in the complex are greater the higher the isoelectric point. This, however, is compensated for by the fact that a high isoelectric point means a high ultimate pH. Soils of this type are probably, therefore, in no greater danger of becoming toxic to plants than are other soils. The isoelectric point and the pH of exchange neutrality were raised by the application of aluminum and lowered through the application of silicate, phosphate, and humus, and the injury point was found to be likewise raised and lowered.

The effect of adding nutrients raised the injury point inasmuch as more of the toxic constituent was brought into solution. Soils having a high manganese content become toxic at a higher pH than when aluminum alone is the cause.

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THE ACCUMULATION AND AVAILABILITY OF PHOSPHORUS IN OLD CITRUS GROVE SOILS

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Although the crop requirements for phosphorus are relatively small, it has been a common practice to apply large amounts of this nutrient to old citrus groves. The reverting and fixing power of the soil for phosphates has been known for many years. Way (10) observed this fact as early as 1850. It is now commonly accepted that the reverting and fixing power of the soil for phosphates is usually in proportion to the content of iron, aluminum, and calcium in the soil. Since these elements occur largely in the finer separates of the soil; namely, silt, clay, and colloidal material, the sands and sandy types of soils would naturally have a low phosphate fixing power.

This would mean that the efficiency of phosphates on sandy soils is greater than on clay and loam soils. These facts are of particular interest to Florida agriculture, because it is generally known that the average citrus soil is low in the phosphate fixing agents, iron, etc. Most of these soils are technically classed as sands, and it appears that the high rate of phosphate fertilization (1,500 to 3,000 pounds of superphosphate per acre per year) is not necessary. The object of this study was to determine the availability of the accumulated phosphates in the old citrus grove soils of Florida.

TOTAL AND AVAILABLE PHOSPHORUS IN CITRUS SOILS

The study consisted at first in determining the relation between the total and available phosphorus in representative soils (from seedling groves 45 to 65 years of age, and budded groves from 4 to 27 years of age). For comparison, samples of virgin soils nearby each grove were studied. The samples were obtained by compositing four separate portions (borings) each for the first, second, and third foot depths of soil. This was done with considerable precaution in order to avoid irregular samples resulting from previous fertilizer practice. The samples were brought to the laboratory, dried, and analyzed for total and available phosphorus, using standard methods of procedure (Methods of Official Analysis Association Official Agricultural Chemists, 1925, for total; and 0.002 N sulfuric acid for available phosphorus (9). The results are given in table 1. From this table it will be seen that the total, as well as the available, phosphorus increases with the age of the grove. This would be expected in view of the common practice, especially regarding the total phosphorus. It is interesting also to note that the total phosphorus is greater in the virgin soils near the seedling groves than those near the young groves.

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TABLE 1

The relation between total, available, and water-soluble phosphorus in citrus grove soils

SOIL TYPE	AGE OF GROVE	TOTAL P_2O_5		AVAILABLE P_2O_5		WATER-SOLUBLE PHOSPHORUS 1–5 EXTRACT	
		Grove	Virgin	Grove	Virgin	Grove	Virgin
		per cent	*per cent*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Blanton fine sand	Old seedlings	0.211	0.037	440	66.0	12.0	tr
Blanton fine sand	Old seedlings	0.170	0.036	273	52.8	16.5	tr
Norfolk fine sand	Old seedlings	.....	.....	352	26.4	11.1	tr
Norfolk fine sand	Old seedlings	0.284	0.018	264	33.0	7.5	1.6
Norfolk fine sand	Old seedlings	0.350	0.052	401	70.4	7.3	tr
Orlando fine sand	Old seedlings	0.342	0.033	396	19.8	15.0	tr
Orlando fine sand	Old seedlings	0.275	0.100	275	35.2	11.2	2.0
Gainesville fine sand	Old seedlings	0.391	0.110	440	66.0	15.0	tr
Norfolk sand	Old seedlings	0.275	0.024	279	66.0	14.1	tr
Norfolk sand	Old seedlings	0.561	0.021	528	44.0	15.6	tr
Average of old seedling groves (45–65 years)		0.317	0.048	364.8	47.9	12.5	0.36
Norfolk fine sand	18 years	0.105	0.051	158	70.4	....	....
Norfolk fine sand	18 years	0.144	0.040	378	30.8	4.0	1.0
Norfolk fine sand	20 years	0.150	0.057	250	136.0	6.2	tr
Norfolk fine sand	18 years	0.170	0.042	178	26.4	9.0	tr
Norfolk sand	27 years	0.361	0.056	211	44.0	10.1	5.3
Lakewood sand	27 years	0.240	0.036	272.8	26.4	15.5	tr
St. Lucie sand	15 years	0.025	0.012	136.4	tr	6.0	tr
Norfolk sand	16 years	0.079	0.021	440.0	tr	6.5	tr
Average of budded groves		0.159	0.039	253	41.7	8.1	0.90
Norfolk sand	7 years	0.058	0.021	53.8	15.4	2.0	tr
Norfolk sand	8 years	0.091	0.035	55.0	14.0	1.2	tr
Norfolk fine sand	4 years	0.074	0.056	72 6	50 6	tr	tr
Norfolk sand	5 years	0.029	0.033	121.0	70.4	3 2	tr
Norfolk sand	10 years	0.019	0.033	59.4	14.0	0.1	tr
Lakewood sand	10 years	0.061	0.050	105 6	55.0	2.0	tr
Norfolk sand	8 years	0.030	0.017	70.4	66.0	tr	tr
Norfolk sand	5 years	.....	.....	22.2	22.0	tr	tr
Norfolk sand	7 years	.....	.....	22.7	tr	tr	tr
Blanton fine sand	8 years	.....	.....	39.6	11.0	3.0	tr
Average of young groves (4–10 years)		0.051	0.035	62.2	35.3	1.9	tr

This is apparently because the seedling groves are located on better soils than are the young groves.

The old seedling grove soils have an average phosphate accumulation equivalent to over 60,000 pounds of superphosphate (16 per cent) per acre foot. In view of the high rate of fertilizer applications to citrus (over 2,500 pounds of fertilizer containing 8 per cent available phosphoric acid per acre per year) this accumulation can be accounted for. The content of available phosphorus in the soils from the old seedling and budded groves is remarkably high compared to that of the virgin and young grove soils. The high content of available phosphorus in the older groove soils indicates that the soils had not fixed (reverted) all of the applied phosphates. On this assumption, the water soluble phosphorus in these soils would be higher than that reported for the average soil in humid regions. In order to determine whether or not this assumption was true, water extracts were made by shaking one part of soil with five parts of water for 25 minutes. The solutions were then filtered and the phosphorus was determined in the filtrate. These results also are given in table 1. Here the content of water-soluble phosphorus increases with the age of the grove, the old seedling groves having an average of 12.4 p.p.m. of phosphorus, or 24.8 pounds per acre 6 inches of soil. This is equivalent to about 300 pounds of 16 per cent superphosphate and considerably more than the average crop will absorb. When compared with the virgin soils these results are very high.

PHOSPHORUS IN THE SOIL SOLUTION

The phosphorus concentration of the soil solution was determined in a number of grove soils, using the following methods: 400 gm. of soil was placed in a 2 by 12 inch cylinder and compacted. Distilled water was then added to the soil columns until the soil was thoroughly moist. Additional water was added to displace the solution from the columns of soil. The phosphorus was determined in the displaced solution of 50-cc. portions. As a means of comparison, a heavily fertilized cotton soil from Alabama,[2] a celery soil from Sanford, Florida; and two virgin soils were included in the study. The results are given in table 2. Only traces of phosphorus were found in the virgin and cotton soils. But the seedling grove and celery soils contained a rather high concentration of phosphorus in the soil solution. Moreover, the concentration increased with successive leachings. This would be expected because of the removal of the soluble calcium (reverting agent) more rapidly than the phosphorus.

Another group of soils was studied in regard to the leachable phosphorus. In this case 10 gm. of soil was leached with 10-cc. portions of water for 25 times, and the phosphorus determined in each leaching. Here the virgin soils as well as heavily fertilized cotton soils[2] were included for comparison.

The results as shown in table 3 are surprising in that the grove soils contain a remarkably high content of leachable phosphorus. Although the quantity in solution at a given time is small, the total amount leachable is large. Many of the grove soils contained over 50 parts per million of leachable phosphorus.

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[2] The author is indebted to Drs. J. W. Tidmore and W. A. Albert for furnishing the soil samples from the Alabama Experiment Station and the South Carolina (Pee Dee) Experiment Station used in this study.

These results agree with the high content of water soluble phosphorus (1–5 soil water extract) shown in table 1. That the phosphorus actually leaches from the soil is of unusual interest, because this nutrient is not considered to be leachable. Some of these same soils were leached with a dilute acid (0.002 *N* H_2SO_4) and the phosphorus determined in the leachate with the results that the

TABLE 2

Concentration of phosphorus in the soil solution of old seedling groves

SOIL TYPE	KINDS OF PLANTS	PARTS PER MILLION PHOSPHORUS IN SOIL SOLUTION, DISPLACED BY SUCCESSIVE LEACHINGS OF 1 INCH WATER (50 CC.)					
		1st	2nd	3rd	4th	5th	6th
Norfolk fine sand..........	Seedling orange	4.1	5.0	9.0	10	14	13
Norfolk fine sand..........	Forest-virgin	tr	tr	tr	tr	tr	tr
Norfolk fine sand..........	Seedling orange	7.7	7.1	10	15	18	22
Norfolk sand..............	Seedling orange*	4.4	3.1	4.0	4.3	3	4
Norfolk sand..............	Seedling orange	3.0	5.0	7.0	15	18	18
Gainesville fine sand.......	Seedling orange	1.8	2.3	2.9	3	5	5
Leon fine sand............	Celery for 20 years	4.6	6.2	15	24	28	31
Orlando fine sand..........	Seedling orange	8.0	7.0	15	20	16	23
Parkwood clay loam.......	Forest-virgin	tr	tr	tr	tr	tr	tr
Norfolk sandy loam........	Cotton High Fert.†	0.3	0.5	tr	tr	tr	tr

* No phosphorus applied to soil for 3 years previous of taking samples.

† Heavily fertilized cotton soil from Alabama.

TABLE 3

Leachable phosphorus from old citrus grove soils

SOIL TYPE	KIND OF CROP	PARTS PER MILLION PHOSPHORUS (IN AIR-DRY SOIL) IN SUCCESSIVE LEACHINGS OF 10-CC. PORTIONS								
		1st	2nd	3rd	4th	5th	10th	15th	25th	Total of 25 leachings
Blanton fine sand........	Seedling grove	2.2	2.5	3.1	3.1	2.2	4.0	4.1	2.7	58.7
Gainesville fine sand......	Seedling grove	1.5	2.0	2.2	2.1	1 4	3.6	2.5	1.2	50.3
Norfolk sand............	Virgin	tr	tr	tr	tr	tr	tr	tr	tr	tr
Norfolk sand............	Truck for 25 years	3.6	3.4	3.6	3.4	2.2	3.4	3.1	0.7	65.5
Norfolk sandy loam*......	Cotton (about 25 years)	0.2	tr	tr	tr	tr	tr	tr	...	0.2
Norfolk fine sand.........	Seedling grove	3.9	4.0	4.1	3.9	2.7	3.5	3.6	3.1	59.8
Norfolk fine sand†........	Seedling grove	2.5	3.1	3.5	3.4	2.8	3.4	2.0	2.2	54.8
Norfolk fine sand.........	18-year grove	2.1	2.4	2.7	2.6	2.2	3.0	1.2	1.7	42.8
Norfolk fine sand.........	Virgin	tr	tr	tr	tr	tr	tr	tr	tr	tr
Orlando fine sand........	Seedling grove	1.2	1.7	1.7	1.3	1.1	2.8	3.0	0.7	32.2
Ruston fine sandy loam‡...	Cotton (about 18 years)	0.2	0.17	tr	tr	tr	tr	tr	tr	0.37

* Heavily fertilized cotton soil from Alabama Experiment Station.

† No phosphorus applied to soil 3 years previous to taking samples.

‡ Heavily fertilized cotton soil from South Carolina Experiment Station.

old seedling groves soils contained over 500 pounds of dilute acid soluble phosphorus per acre.

The results in table 3 indicate that the surface soils from the old groves have become partially saturated with phosphorus, and are thus subject to the influence of percolation water. In order to determine whether or not any of the applied phosphorus had been translocated by percolation water, the available phosphorus was determined in the first, second, and third foot depths of 10 seedling grove soils, 9 groves 13 to 30 years old, and 5 groves 2 to 6 years old, as well as virgin soils. The average results are given in table 4.

These data show that the phosphorus in the 3-foot level of the old groves soils was almost four times that in the virgin soils. It is evident that some of the applied phosphates had been translocated to the second and third foot levels in the old grove soils, but not in the young grove soils. Evidently the young grove soils had not become saturated with phosphates.

TABLE 4

Available phosphorus at different depths of citrus soils

AGE OF GROVES	AVAILABLE PHOSPHORUS IN SOIL					
	Grove soil			Virgin soil		
	A*	B†	C‡	A*	B†	C‡
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Average for old seedling groves	173	76.1	47.9	26.1	13.4	12.0
Average for intermediate age groves	107.8	42.2	2.14	17.3	7.5	5.1
Average for young groves	22.8	9.0	7.0	10.4	13.8	6.0

* A = 0 – 12 inches.
† B = 12 – 24 inches.
‡ C = 24 – 36 inches.

FIXATION OF PHOSPHORUS BY CITRUS SOILS

The preceding results indicate that the citrus soils have a low phosphate precipitating or fixing power. This is suggested by the relatively low content of iron and other fixing agents in the sandy soils. To obtain positive information on this subject, the phosphate fixing power of a number of representative soils was determined in the following manner: 400-gm. portions of soil were compacted in 2 by 12 inch cylinders. The columns of soils were then leached daily with 100 cc. of a solution containing 100 p.p.m. of phosphorus (in monocalcium phosphate). The leachates were analyzed for phosphorus each day until the solutions came through the column of soils unchanged. The soils were allowed to dry in the cylinders and again leached with the phosphorus solutions, with the results that drying somewhat imcreased the amount of phosphates fixed.

The total amount of phosphorus fixed by the soils was calculated in terms of superphosphate and the result is included in table 5. It will be seen that two soils fixed phosphorus equivalent to over 6,000 pounds of superphosphate per acre 6 inches. The others fixed less than 5,000 pounds per acre. Two of the soils fixed less than 1,000 pounds, both of which were sands.

TABLE 5

Absorption of phosphorus by citrus soils

(Determinations made by passing a solution of phosphorus through a column of soil)

SOIL TYPE	SUPERPHOSPHATE ABSORBED PER 2,000,000 POUNDS OF SOIL
	pounds per acre
Blanton fine sand	8,580
Leon sand	200
Norfolk fine sand	3,206
Norfolk sand	2,709
Norfolk sand	3,975
Norfolk sand	4,664
Norfolk sandy loam	6,309
Norfolk sand	3,498
Norfolk sand	4,054
Norfolk sand	980
Orlando fine sand	3,365
Orlando fine sand	4,081

PLANT RESPONSE TO ACCUMULATED PHOSPHATES

Although laboratory studies show a high content of available and water-soluble phosphorus; the final test is that of plant response. Does the plant respond to added phosphorus when the soil contains a high content of water-soluble phosphorus? If the laboratory studies are confirmed by plant responses then the results become more reliable. If a soil contains 50 pounds of water-soluble phosphorus per acre, and the crop requires only 10 to 15 pounds, would plant responses be obtained from additional phosphates? Answers to these questions are of practical as well as scientific value.

The plant responses to the accumulated phosphates, was studied by diluting four parts of the soil from an old seedling grove with six parts of clean quartz sand. These mixtures were then placed in glazed earthenware jars and treated according to table 6. The cultures were then planted to rye and the seedlings allowed to grow to maturity, water being added from time to time as needed. After the rye was harvested the soils were again mixed and planted to mustard, which was allowed to grow and likewise to sorghum and soybeans, water and nutrients, as indicated in table 6, being added as needed. Calcium sulfate was added at the rate of 1 ton per acre before the soybean crop was planted. The dry weights of these crops are given in table 6. The results show no consistent evidence of stimulation to added phosphorus. The differences between cultures 3 and 4 are too small to be significant so far as phosphorus is concerned. However, cultures 2 and 5 of the sorghum crop showed distinct potash deficiency. All the crops showed a decided response to nitrogen. Although the soybean crop made a slightly higher total yield with added phosphates, the amount of seed produced was greater without added phosphates.

The relative growth of these crops is given in plates 1 and 2.

The available and water-soluble phosphorus (1–5 extract) in these soils were determined after the soybean crop was harvested. The results are given in table 6. It is interesting to note that the available and water-soluble phosphorus, although variable, was less in many of these cultures than that of the soils under field conditions. This is due, no doubt, to the dilution of the soils with sand. Furthermore, small amounts of the cultures (1 kilo) were used, and the plants depleted the soil of the original phosphorus present.

TABLE 6

The availability of phosphates in old citrus grove soils as measured by annual plants and laboratory methods

NO.	SOIL TREATMENT	FERTILIZER ADDED	WEIGHT OF CROPS (OVEN-DRY)				AVAILABLE PHOSPHORUS§	WATER-SOLUBLE PHOSPHORUS§
			1st crop Rye	2nd crop Mustard	3rd crop Sorghum	4th crop Soybeans		
			gm.	*gm.*	*gm.*	*gm.*	*p.p.m.*	*p.p.m.*
1	Original soil	None	6.37	0.4	1.67	1.5	85	0.7
2	400 gm. soil, 600 gm. sand	N*	6.77	3.55	4.05	9.65	94	tr
3	400 gm. soil, 600 gm. sand	N, K†	8.91	4.80	11.80	9.90	70	tr
4	400 gm. soil, 600 gm. sand	N, P,‡ K	8.95	5.20	11.51	10.70	129	0.7
5	400 gm. soil, 600 gm. sand	N, P	7.05	3.90	5.00	8.95	170	0.9
6	400 gm. soil, 600 gm. sand	P, K	1.60	0.10	1.80	3.45	228	1.1
7	400 gm. soil, 600 gm. sand	P	1.72	0.0	1.10	3.85	158	0.75
8	400 gm. soil, 600 gm. sand	K	1.71	0.2	1.40	4.45	110	0.42
9	400 gm. soil, 600 gm. sand	None	1.69	0.0	2.30	3.60	106	0.40

Fertilizer added:

* N—nitrogen, One-half gram sodium nitrate was applied to rye, ⅓ gm. calcium nitrate to mustard, and ⅓ gm. ammonium nitrate to sorghum, where cultures received nitrogen.

† K—Potash, One-eighth gram sulfate of potash was applied to all crops where cultures received potash (except No. 8 sorghum).

‡ P—Phosphorus, One gram 16 per cent superphosphate was applied to rye crop and 1 gm. to sorghum crop (except No. 7) where cultures received phosphorus. One gram calcium sulfate was applied to all cultures before soybean crop.

§ Available and water-soluble phosphorus determined after soybean crop.

Another series of cultures was prepared in which larger vessels were used (6-gallon jars and one part soil with three parts quartz sand) and set to sour orange seedlings. The cultures were treated according to table 7 and the seedlings allowed to grow for 10 months. The seedlings were measured (diameter of trunk) when set and again at the same mark after 10 months. The growth records of the seedlings are given in table 7. Here the dilution of the soil did not reduce the phosphorus concentration below the optimum growth concentration for citrus seedlings, as indicated by figure 2 of plate 2. Thus far no evidence of phosphorus deficiency has been observed.

The seedlings without added phosphorus were apparently as good as those receiving phosphorus. Yet the phosphorus in the cultures receiving only nitrogen and potash is much less than that in grove soils. This is due to dilution and the fixing power of the sand. The results dealing with plant responses seem to confirm the laboratory findings regarding the availability of the phosphorus in the old grove soils.

TABLE 7

The influence of accumulated phosphate in old citrus grove soils on the growth of citrus seedlings

SOIL ¼ PART, SAND ¾ PART	TREATMENT OF SOIL	DIAMETER OF SEEDLINGS			AVAILABLE PHOSPHORUS	WATER-SOLUBLE PHOSPHORUS
		July 21, '31	May 11, '32	Gain		
		mm.	*mm.*	*mm.*	*p p.m.*	*p.p.m.*
1	N* only	80	125	45		
2	N* only	94	127	33		
3	N* only	68	109	41	35	0.66
		Total gain...........		119		
4	N, K	95	137	42		
5	N, K	65	108	43	38	0.65
6	N, K	74	109	35		
		Total gain...........		120		
7	N, P, K	60	105	45		
8	N, P, K	73	116	42	120	1.75
9	N, P, K	80	116	33		
		Total gain...........		120		

* N—Nitrogen in calcium nitrate; K—potassium sulfate; P—superphosphate.

DISCUSSION

Although the results reported herein are somewhat different from that suggested by field practice they seem to confirm the work of Stephenson and Chapman (8) dealing with the penetration of phosphates in California soils. There is little doubt about the heavily fertilized soils of Florida containing a high content of water-soluble phosphorus. This is confirmed by leaching and plant response studies. It is admitted that the movement of phosphorus in California soils is less than that in the soils of Florida. This is to be expected because Florida soils are lighter and contain a smaller quantity of fixing agents than the California soils. According to Babcock and his co-workers (2, 8) in soils having as much as 2 p.p.m. of phosphorus in the soil extract (1 to 5 water), no response to citrus is likely to occur with added phosphorus.

This is in agreement with the results of other investigators dealing with annual plants (6).

Inasmuch as citrus is a perennial plant having a deeper and more extensive root system than annuals, this crop should not require any higher concentration of phosphorus than, if as high as, do annuals. Pierre and Parker (6) reported that the water-soluble phosphorus was very low in most of the humid soils. They found less than 1 p.p.m. of phosphorus in the soil solutions investigated. The old grove soils of Florida contain 8 to 15 p.p.m. of phosphorus in the soil solution, as indicated in tables 1 and 2. In all probability the phosphorus concentration of the apple and peach soils in other states is less than one-tenth that of the heavily fertilized grove soils of Florida. According to Cooper (3) even with low concentration of phosphorus in the soil solution, no striking stimulation to phosphate fertilizers has been reported for apples and peaches.

Fraps (5) of Texas reported that phosphates percolate through certain sandy soils readily, but apparently little significance was given to his work.

Anderson (1) and his co-workers found a rather high accumulation of phosphorus in the old tobacco soils of Connecticut with a large percentage in an available form. There studies indicate that it is not necessary to add a high proportion of phosphorus to the old tobacco soils for optimum production. This is of interest because the tobacco soils very likely have a higher phosphate fixing power than the citrus soils of Florida. It seems plausible to assume that the accumulated phosphates in Florida soils would be more available than would be those in the tobacco soils of Connecticut.

It is extremely doubtful that citrus would suffer if the proportion of phosphoric acid was reduced to one-half that of nitrogen, especially in old groves. The fact that the plants including citrus seedling showed no material response to added phosphorus under controlled conditions indicates that the optimum phosphorus concentration of the soil solution is much less than that of the old citrus grove soils. Floyd (4) was not able to find any response to citrus seedling in virgin soils from added phosphates over a period of 2 years. This would indicate that citrus does not require a high concentration of available phosphorus for optimum growth. It is improbable that the virgin soils in his studies contained much water-soluble phosphorus.

No claim is made for the optimum concentration of phosphorus in the soil solution for maximum citrus production. This is not definitely known, but the fact that this crop grows successfully on marl and heavy clay soils, which are known to have a low content of water-soluble and available phosphorus, indicates that the requirements are not as high as that in the grove soils reported in this paper. This is further confirmed by the fact that the annual crops did not respond to added phosphorus even where the concentration was less than that of the old grove soils.

It is possible that the citrus groves in Florida are benefited by heavy applications of superphosphate. Certainly the results of this investigation indicate that much more is being applied than is required by the trees. However, there is a possibility that the superphosphate may have positive indirect effects which are not yet well understood.

It is interesting to note that Floyd (4) found no consistent difference in growth and yield of citrus with a phosphoric acid variation ranging from 2 to 12 per cent over a period of 4 years. According to Floyd this lack of difference is probably due to the reserve supply of phosphoric acid in the soil resulting from previous fertilizer treatments. It would be logical to assume that the soils receiving the 2 per cent phosphoric acid had a much less supply of available and water-soluble phosphorus than the soils from groves fertilized in the usual way. Floyd's results are confirmed by the laboratory and green house studies reported in this paper.

SUMMARY

The total, available, and water-soluble forms of phosphorus were determined in the soils from old seedling and budded citrus groves of different ages, as well as in virgin soils taken near the groves. The results show that the total, available, and water-soluble phosphorus increases with the age of the groves. Although the accumulated phosphorus is not unusually high, the available and water-soluble phosphorus is very high compared to that of soils in other humid regions. The concentration of phosphorus in the soil solution was also found to be high compared to other humid soils. The phosphate fixing power of Florida sandy soils was also determined. The results show that these sandy soils have a low phosphate fixing power—500 to 6,000 pounds of superphosphate per acre 6 inches. Plant response studies (annual crops and citrus seedling under controlled conditions) showed no stimulation from added phosphates on soils from old seedling grooves. All of the data tend to indicate that the phosphate requirements of the citrus soils (sands) of Florida are much less than the common practice would indicate.

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PLATE 1

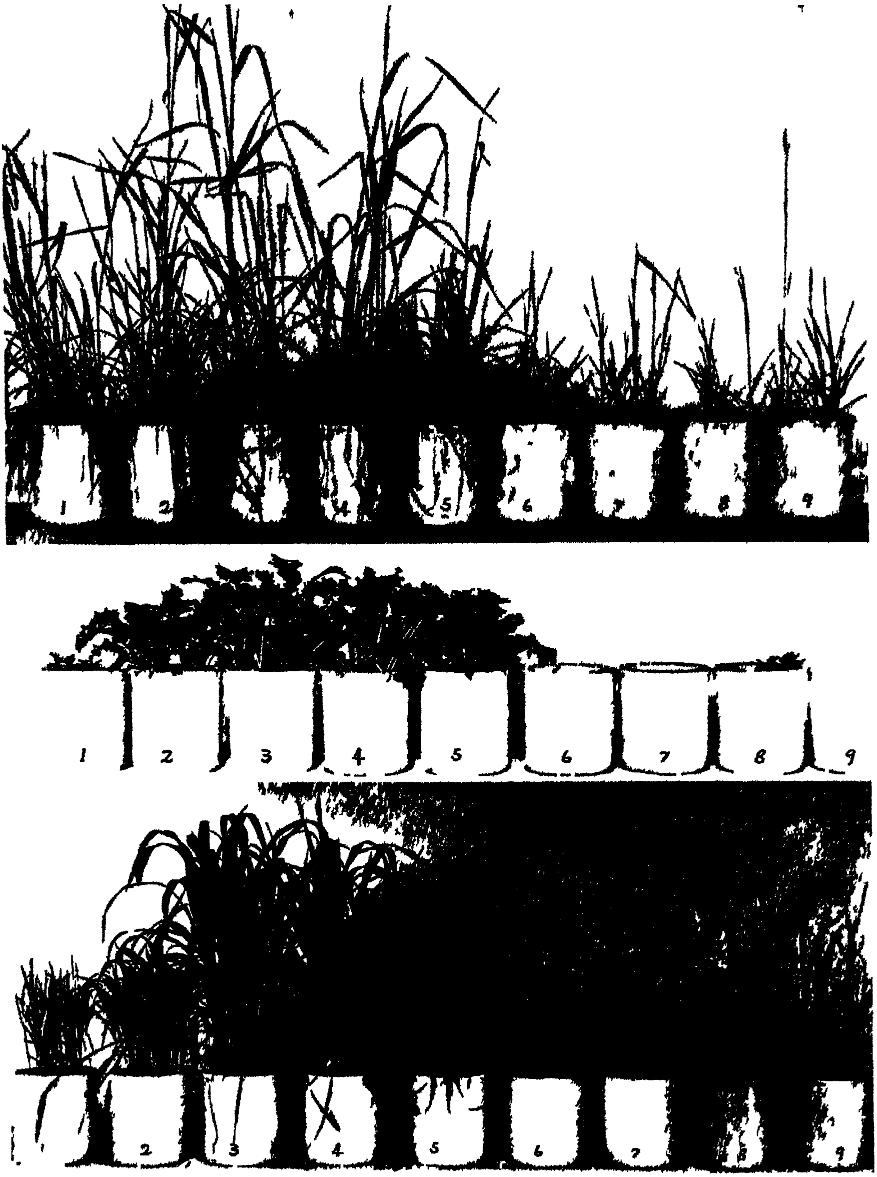
RESPONSE OF RYE, MUSTARD AND SORGHUM TO THE ACCUMULATED PHOSPHATES IN OLD CITRUS GROVES

FIG. 1. Rye.

FIG. 2. Mustard.

FIG. 3. Sorghum.

Culture 1, original soil without fertilizer; cultures 2–9 inclusive, 4 parts soil and 6 parts sand; Culture 2 received nitrogen only; 3, nitrogen and potash; 4, nitrogen, potash and phosphorus; 5, nitrogen and phosphorus; 6, phosphorus and potash; 7, phosphorus; 8, potash; 9, nothing. See table 6 for yields.

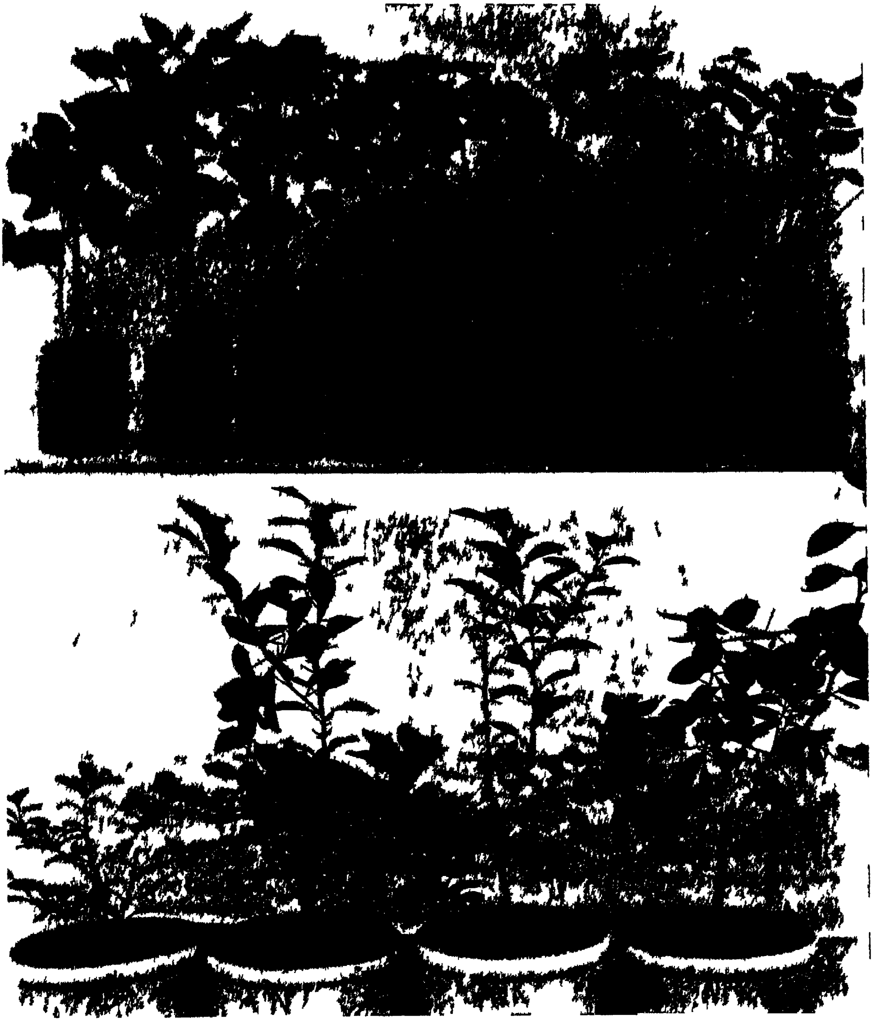


FIGS 1–3

THE RESPONSE OF SOYBEANS AND ORANGE SEEDLINGS TO THE ACCUMULATED PHOSPHATES IN OLD CITRUS GROVE SOILS

FIG. 1. Soybeans. See plate 1 for treatments.

FIG. 2. Orange seedlings. One-fourth soil and three-fourths quartz sand. See table 7 for growth.



No treatment | Nitrogen only | Nitrogen and potassium | Nitrogen, potassium and phosphorus

FIGS 1 AND 2

SOME FACTORS LIMITING THE APPLICABILITY OF BIOLOGICAL METHODS FOR DETERMINING THE AVAILABILITY OF PLANT FOOD ELEMENTS IN CALCAREOUS SOILS[1]

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In a previous paper (4) it was shown that the Azotobacter (plaque) method is not generally suitable for determining the availability of phosphate or potassium in Arizona soils. It is believed that the limitations of the method can possibly be explained on the basis of developments brought out in recent studies on phosphate availability in Arizona soils.

The soils of Arizona are characterized by the presence of varying amounts of calcium carbonate, very small amounts of organic matter, and appreciable amounts of phosphorus. Many of the soils, except black alkali types, which are always well supplied with soluble forms, yield water extracts containing only minute amounts of phosphorus. The electrodialyzable phosphate greatly exceeds the amounts given by productive eastern soils yet these same soils show all indications of phosphate deficiency and respond to phosphate fertilization.

Further studies by Zscheile (13), McGeorge, Breazeale, and Buehrer (1, 2, 5, 6, 7, 8) have demonstrated that the ion (H_2PO_4) is dominant in plant sap, that it is present in largest amounts in acid soils, and that crops subsist on lower concentrations of phosphate in the soil solutions of slightly acid soils. Hence, it appears that plants absorb H_2PO_4 ion for nutrition purposes rather than HPO_4 or PO_4. This assumption has been supported by culture experiments which have demonstrated that plants are not able to absorb phosphate or nitrate ions at greater alkalinity than approximately pH 7.6, yet water intake is not impaired (1).

The relative proportions of the three phosphate ions at different pH values as calculated by Buehrer (2, 5) are of interest in this connection and are given in table 1.

These data show that at pH 6.8 the amounts of H_2PO_4 and HPO_4 are approximately the same; below pH 6.8, H_2PO_4 predominates, whereas at high pH values HPO_4 is in excess. This, in turn, explains why plants may suffer from phosphate starvation in soils which contain appreciable quantities of soluble phosphorus.

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[1] Contribution from the department of agricultural chemistry, Arizona Agricultural Experiment Station.

Further studies have also shown that nutritional disorders in plants and phosphate availability in soils are closely connected with the presence or absence of carbon dioxide (1, 7).

The author has reported (4), in a study of the Azotobacter (plaque) method of determining phosphate availability, many cases of soils containing an active Azotobacter flora, as evidenced by growth and vigorous nitrogen fixation in Ashby's solution, which did not develop colonies on the plaques under any treatment.

Breazeale and McGeorge (1) found that although a plant can not absorb phosphate from solution cultures at high pH, it will absorb phosphate if the pH of the solution is lowered by introducing CO_2. The plant is able to exude CO_2 from its roots and thus lower the pH of the soil in the root-soil contact zone, thereby permitting phosphate intake. Now if a dispersed soil be aerated with CO_2 (assisted in field conditions by adding gypsum, sulfur, or organic matter) the pH of the soil-root zone is lowered, and the insoluble soil phosphorus, which is present as a carbonate phosphate compound consisting of a 1 mol $CaCO_3$ and 3 mols $Ca_3(PO_4)_2$, is rendered more soluble by the CO_2 evolved (7).

TABLE 1

Concentration of phosphate ions (mgm.) in a solution containing 1 mgm. per liter

pH	H_3PO_4	H_2PO_4	HPO_4	PO_4
4	0.0091	0.99	0.0019	7.0×10^{-13}
5	0.00089	0.98	0.0194	$6\ 9 \times 10^{-10}$
6	0.00076	0.79	0.164	5.9×10^{-8}
7	3.1×10^{-6}	0.33	0.66	2.4×10^{-6}
8	$4\ 4 \times 10^{-8}$	0.048	0.94	3.4×10^{-5}
9	4.6×10^{-10}	0.0049	0.98	3.5×10^{-4}

Sackett and Stewart (11) recommend in the Azotobacter method the addition of $CaCO_3$ to acid soils, the neutralization of basic soils with H_3PO_4, and the aeration of "adobe" or heavy soils by the addition of sand. The principal objection to laboratory methods of determining soil fertility is the use of conditions very remote from those found in the field. If a "black alkali" soil or a soil of the adobe type (pH 7.8 – 8.0) is tested according to the method of Sackett and Stewart, without neutralization or aeration, no colonies are formed, as has been already demonstrated (4). This is because at this pH the amount of H_2PO_4 is at a minimum; and if H_3PO_4 be added, particularly in small amounts, it is converted in the presence of solid phase $CaCO_3$ to insoluble "carbonato-phosphate." Azotobacter is an obligate aerobe, and the types of soils mentioned tend to become deflocculated upon the addition of water. Under these anerobic conditions, the Azotobacter does not develop and there is not sufficient CO_2 present to reduce the pH enough to permit absorption of H_2PO_4. Reducing the pH or aerating serves to produce conditions in the soil favoring the intake of phosphate by Azotobacter (or plants) and such artificial conditions, therefore, *are only an index of soil treatments necessary for phosphate assimilation.*

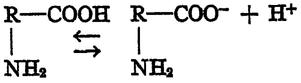
In the foregoing statement, it is assumed that Azotobacter assimilates phosphorus in the form of H_2PO_4. This must be the case if Azotobacter is to serve as an index of phosphate availability for higher plants. The optimum reaction for Azotobacter is usually assumed to be from pH 6.8 to 7.8, and the extreme range from approximately pH 6.0 to 8.8. The author (4) has found fixation by Azotobacter in soils of pH values as high as pH 9.0, 9.5, and 10.0. It is questionable, however, that the Azotobacter was subjected to these values any more than the growing root of a plant in the same soil. Carbon dioxide is recognized as one of the principal products of the metabolism of Azotobacter, and according to Waksman (12) there is also a close relation between CO_2 production and nitrogen fixation. Hence, there was probably sufficient CO_2 produced to lower the pH sufficiently to permit assimilation of H_2PO_4.

In order to determine whether Azotobacter is able to reduce the pH as described in the foregoing, the following experiment was conducted: sterile flasks of Ashby's solution were inoculated in duplicate with a suspension of *Azotobacter agilis*, a third flask being left uninoculated to serve as a control. The results were:

Original pH	*pH after 6 days*	*Mgm. N fixed per gm. mannite*
5.3	5.3	0
6.0	5.7	2.0
7.1	6.0	2.8
7.7	6.0	4.0
8.0	6.0	4.0
9.0	6.0	3.0

Gainey and Batchelor (3) have demonstrated that from pH 5.5–6.0, little fixation of nitrogen resulted; from pH 6.3 to 7.2, fixation was approximately the same, but was vigorous at pH 6.1–6.5. These results show, therefore, that H_2PO_4 is the preferred form of phosphate by Azotobacter as well as plants, and that they are able to reduce higher pH values by production of CO_2 and organic acids.

Buehrer (2) has calculated from the thermodynamic free energy equation for the reversible transfer of 1 mol of phosphate from soil solution to plant sap, the energies of absorption of phosphate at various pH values. These energy requirements are minimum between pH 4.0 and 6.8. Accordingly, greatest fixation should occur at lower pH values, which is in agreement with the discussion in the preceding paragraphs. It is probable that at about pH 6.0, the Azotobacter cells are brought to their isoelectric point, thereby prohibiting the intake of ions. Apparently the isoelectric point of Azotobacter has never been determined, but some preliminary experiments employing the staining technique of Naylor (9) have indicated that Azotobacter might have a rather wide isoelectric range (similar to many plant cells) of approximately pH 4.0 – 6.0. Further studies are now in progress. If this is the isoelectric range of Azotobacter, then it is apparent that growth occurs on the alkaline side. Accordingly positive ions, K, Ca, Mg, etc. are probably taken up by adsorption and by successive ion exchanges, similar to the operation of a "bucket-brigade" as Buehrer has suggested (2). The negative ions would not be taken up in this manner. Buehrer (2) has explained this condition, assuming that the cell membrane is protein, ionizing as follows in alkaline solutions (i.e. pH above isoelectric point).



$$\begin{array}{l}R\text{—}COOH\\ |\\ NH_2\end{array} \leftrightarrows \begin{array}{l}R\text{—}COO^- + H^+\\ |\\ NH_2\end{array}$$

As the hydrogen is neutralized off, the negative acid radical remains in the membrane surface. By virtue of the Helmholtz double layer, the outer surface will have a net negative charge, and the inner surface a net positive charge. The effect of this negative surface is to repel negative ions and prevent their being absorbed. It is possible, however, for the plant to eliminate H and HCO_3^- ions (by way of carbon dioxide) through the mechanism of micelle transfer and thus reduce the pH in the immediate surroundings of the membrane to enable absorption of phosphates to occur.

Breazeale and McGeorge (1) have shown that plants do not absorb phosphates from solution of high pH, but first reduce the reaction to the neutral point, and then absorb phosphates actively. It seems possible that Azotobacter may utilize the same mechanism for phosphate assimilation.

In order to test the validity of this assumption a series of flasks of sterile Ashby's mannite solution of pH 9.0 were inoculated with a suspension of *Azotobacter agilis*, without the addition of calcium carbonate, and incubated at 30°C. Phosphate and pH determinations were made at frequent intervals. The pH dropped from 9.0 to 8.6 in 6 hours, but there was no decrease (i.e. intake) in phosphate. At the end of 30 hours the pH was 7.3 and there was a marked decrease in the phosphate content of the solution, showing that at the lowered pH, phosphate was taken in. At the end of 3 days the pH was 7.0 and there was a further decrease in the amount of phosphate in the solution. It seems probable that Azotobacter uses the same mechanism as plants in its phosphorus intake, namely, reducing the pH of the solution and subsequent intake of phosphorus in the form of H_2PO_4 ion.

SUMMARY

The Azotobacter method for determining availability of soil phosphates has been previously shown to be of little value under the conditions found in Arizona. Although it has given good results in non-calcareous soils, its use, like other chemical and biological tests is limited to the more humid and non-calcareous areas.

The results previously reported are discussed in terms of more recent investigations to show why the method is so restricted in its application. Briefly, the following factors are responsible:

The plant feeds upon H_2PO_4, which occurs in greatest amounts in acid solutions.

The optimum pH range for growth of Azotobacter lies within the range where H_2PO_4 occurs in greatest amounts.

If a soil of high pH is well aerated, Azotobacter will develop and, by the production of CO_2 and organic acids, will lower the pH of the soil solution sufficiently to permit absorption of phosphate.

In "black alkali" soils, the addition of water in the preparation of the plaque results in anerobic conditions. Consequently, Azotobacter cannot develop, and, as a result of the lack of CO_2, the insoluble carbonate-phosphate is not rendered available.

The foregoing conditions have been experimentally and theoretically demonstrated as affecting the availability of phosphates for plants. *The same must be true for Azotobacter, or the test is worthless for determining the availability of nutrient elements for higher plants.*

Under humid conditions, where soils are usually acid and contain more organic matter and CO_2, the phosphate is readily available for plants and for Azotobacter. There is evidence to show that the test for phosphate may be valuable under these conditions, although, as Pittman and Burnham (10) have claimed, the test agrees with field conditions only in the extreme ranges: soils deficient or well-supplied with phosphorus. The author has unpublished data which show that the phosphate requirements for Azotobacter are very small and the relative amounts of nitrogen fixed to phosphate added are not a linear function but act in accordance with the law of the minimum. Consequently, the value of this test is restricted, and in the author's opinion has been most successful in soils which are extremely deficient in phosphate.

In view of these facts, the mere manipulation of a soil by liming, neutralization, or aeration in order to obtain the growth of Azotobacter does not give a valid test for the availability of phosphorus, but merely indicates (with the exception of extremely deficient soils) the cultural process necessary to render available the phosphate already in the soil.

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SOME FACTORS INFLUENCING NITROGEN FIXATION[1]

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The growth and metabolism of microörganisms cultured in soil are often different from the growth and metabolism of the same microörganisms when cultured in synthetic media. This phenomenon has been repeatedly brought to our attention during recent years while nitrogen-fixing microörganisms were being studied. It has been observed that almost invariably fixation is greater where the cultural medium is soil than where it is a synthetic medium (10). Many substances when applied in soil stimulate nitrogen-fixing microörganisms, whereas if used in other cultural media they may be devoid of stimulating powers or may be toxic (7, 8). Moreover, a number of microörganisms obtained from "alkali soils," if cultured in soil, register appreciable gains of nitrogen, whereas, if cultured in synthetic media, they show slight or even no gain in nitrogen (4, 5, 9).

These findings led to the present investigation in which a study is made of physical and chemical factors which may influence bacterial growth in soil but not in standard synthetic media. Certain elements are known to be required in comparatively large quantities by bacteria for their growth and metabolism; others are needed in smaller quantities; whereas still others stimulate when present in minute quantities and may or may not be essential. It is also possible that certain organic substances may be essential for normal growth and metabolism. These may function for the bacteria as do the vitamins for animals or the bios for yeast.

The difference between soil extract and synthetic media for supporting nitrogen fixation is clearly demonstrated in the following experiment in which the nitrogen-fixing powers of five soils were determined. The soils used were: (*a*) College Farm soil, a highly productive calcareous sandy loam, well supplied with the essential elements of plant-food, except nitrogen, which was low; (*b*) College Farm soil to which had been added 0.66 per cent each of sodium chloride, sodium carbonate, and sodium sulfate and which subsequently had been leached with water until the major portion of the soluble salts had been removed; (*c*) Corinne alkali soil, a sandy loam in its native state containing sufficient soluble salts, mainly chlorides, to render it barren; this had been leached with water until the major portion of the soluble salts had been removed; (*d*) Richland Acres alkali soil, a sandy loam containing in its native condition sufficient alkali salts, mainly sulfates, to render it barren; this, likewise, had been leached with water until the major portion of the soluble salts had been removed; and (*e*) Nephi Dry-farm Substation soil, a highly productive heavy clay soil low in organic carbon and nitrogen, high in calcium sulfate and the other essential plant-food elements.

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Varying quantities of the different soils were added to 100-cc. portions of soil extract Ashby's medium (1) and Ashby's medium containing 1.5 per cent mannite. The soil extract medium was prepared by extracting 100 gm. of the specific soil with 1,000 cc. of distilled water and then adding to the extract the regular ingredients of the Ashby's medium. The inoculated solutions were incubated at 28° to 30°C. alongside sterile checks for 21 days, and the total nitrogen was determined by the Gunning-Hibbard method. From four to eight determinations were made for each treatment, and throughout this article average results are reported along with the probable error calculated from the differences in the various determinations according to the formula

$$\text{P.E.} = .67 \sqrt{\frac{\Sigma d^2}{n^2}}.$$

Where the error is ±0.1 to ±0.05, inclusive, it is reported as ±0.1; if below 0.04 it is reported as ±0.0.

TABLE 1

Milligrams of nitrogen fixed in 100 cc. of soil extract Ashby's medium and 100 cc. Ashby's medium each containing 1.5 per cent mannite and inoculated with varying quantities of the different soils

WEIGHT OF SOIL USED	COLLEGE FARM		SYNTHETIC ALKALI		CORINNE		RICHLAND ACRES		NEPHI SOIL	
	Ashby's medium	Soil extract medium	Ashby's medium	Soil extract medium	Ashby's medium	Soil extract medium	Ashby's medium	Soil extract medium	Ashby's medium	Soil extract medium
gm.	*mgm.* N	*mgm.* N	*mgm.* N	*mgm.* N	*mgm.* N	*mgm.* N	*mgm.* N	*mgm.* N	*mgm.* N	*mgm.* N
10	9.0±0.2	9.5±0.1	5.2±0.2	6.0±0.1	3.2±0.3	5.7±0.1	3.9±0.3	4.0±0.1	8.8±0.3	9.5±0.2
10^{-1}	7.0±0.2	10.1±0.4	3.8±0.2	5.0±0.1	2.9±0.4	5.2±0.2	3.8±0.3	3.6±0.1	9.2±0.3	9.6±0.2
10^{-2}	3.5±0.1	9.5±0.2	0	3.8±0.2	0.1±0.2	0.1±0.1	1.2±0.2	3.2±0.2	0	0.7±0.1
10^{-3}	0	0	0	3.4±0.3	0	0	0	2.9±0.4	0	0
10^{-4}	0	0	0	3.4±0.1	0	0	0	1.4±0.3	0	0
10^{-5}	0	0	0	2.5±0.2	0	0	0	0.5±0.2	0	0
10^{-6}	0	0	0	0	0	0	0	0	0	0

Fixation in the soil extract medium is invariably higher than in the Ashby's medium (table 1). The magnitude of the difference is a function of the specific soil and quantity used as the inoculum. The difference in results obtained with the different media is great where the synthetic alkali and the Richland Acres soils are used. It is smaller with the other soils.

The Hiltner and Störmer dilution method for the estimation of the number of specific groups of bacteria is based on this principle. Consequently, one can approximate the number of nitrogen-fixing organisms in these various soils. The College Farm soil is found by both media to contain 10 nitrogen fixers per gram of soil. The synthetic alkali soil as judged by the Ashby's medium contains 1 nitrogen fixer per gram of soil; as judged by the soil extract medium, however, it contains 10,000 nitrogen fixers per gram of soil. The Corinne soil is found to contain 10 nitrogen fixers per gram as determined both by the Ashby's and soil extract media. The Richland Acres soil is found to contain by the Ashby's medium, 10 nitrogen fixers in a gram of soil and by the soil extract medium 10,000 per gram of soil. The Nephi soil would be assigned 1 nitrogen fixer for each gram of soil when determined by the Ashby's medium but 10 per gram when judged by the soil extract medium.

It is highly probable that even the soil extract medium when inoculated with small quantities of soil yields minimum values, for Robertson (24) has shown that growth in a subculture of certain microörganisms, especially protozoa, is stimulated by the presence of other cells of the same type. "Now as a matter of fact, single individuals isolated into volumes exceeding 1 cc. very rarely survive, and failure to reproduce on the part of individuals isolated into cultures exceeding 0.1 cc. in volume is not at all infrequent" (25). It is known to all workers who have attempted to grow individual yeast cells in large volumes of liquid medium that they die, or when they do survive there is a great prolongation of the lag period (18, 25). "Allelocatalysis," as Robertson terms this phenomenon, is not often manifest by bacteria under ordinary conditions, according to Buchanan (15), yet single cell isolation with some forms, for example, pneumococci, is extremely difficult. This may be due, according to Valley and Rettger, to a low carbon dioxide tension (28).

TABLE 2

Milligrams of nitrogen fixed by Azotobacter chroococcum *in 100 cc. Ashby's and soil extract media during 21 days' incubation*

The initial inoculation varied

NUMBER OF CELLS INOCULATED INTO 100 CC. OF MEDIUM	MGM. OF NITROGEN FIXED IN 100 CC.	
	Ashby's medium	Soil extract medium
1–4	1.9±0.4	3.1±0.6
4–9	1.9±0.1	6.0±0.2
11–15	2.1±0.1	6.6±0.2
43–60	3.8±0.1	6.0±0.2

Azotobacter chroococcum was tested for the "allelocatalytic" reaction by inoculating varying numbers into 100 cc. of Ashby's and soil extract media. After 21 days' incubation at 28° to 30°C., they were analyzed for gains in nitrogen. The average results are given in table 2.

Where from 1 to 4 cells are inoculated into Ashby's or soil extract media the gains of nitrogen are relatively smaller than when larger inoculations are used.

An inoculation of from 4 to 9 cells is just as effective in the soil extract medium as is a larger inoculation. However, this is far from being the case in the Ashby's medium where conditions are not so suitable for growth. Where small inoculations are used the probable error is great. This is due to the variation in the lag period which would be correspondingly smaller as we pass from 1 to 4 cell inoculations.

It is evident that there is something in the soil extract not occurring in the Ashby's medium which favors the growth of *Azotobacter chroococcum*. Just what is it? Krzemeniewski (17) found nitrogen fixation by *Azotobacter chroococcum* decidedly increased by soil humus, either as free humic acid or as salts of potassium, sodium, or calcium. This has been attributed to the iron, aluminum, and silicic acid supplied to the microörganisms through the humus (16). This may be true in part as the fixation varies with the humus. Artificial humus, prepared by boiling sugar with acids, fails to stimulate.

Söhngen (27) found that colloidal iron oxide, aluminum oxide, and silicon oxide all greatly increase nitrogen fixation by *Azotobacter chroococcum*. A similar stimulation was obtained when strips of filter paper or cotton cloth were hung into the medium. He attributes the stimulation to the absorption of oxygen and nitrogen by the colloids. This he maintains would make them more available to the microörganisms. The boiling of natural humus with hydrochloric acid may either remove the foreign material or change it from the colloidal state, and this he found to be the case, rendering it inert. Löhnis and Green (20), take exception to this explanation, for they found no absorptive action exerted by humus either on the nitrogen or the oxygen. Rosing (26) found he could stimulate just as effectively with iron as with humic acid, provided appropriate quantities of iron were used (2, 3). The extent of stimulation varies with the form in which the iron is applied and is most effective as the hydroxide and in the presence of cane sugar. Carefully purified humates apparently do not possess this property (21); it is possessed, however, by the aqueous extract and the phosphotungstic fraction of the aqueous extract of "bacterized peat." The action of the colloid has also been attributed to its catalytic effect, to its protective action against poisons (29), to its buffering action upon the pH of the medium, and to the form in which the phosphorus is held for the metabolism of the Azotobacter (16). Considerable evidence has been produced within recent years which points to a growth-promoting substance which is especially effective toward Azotobacter (13, 14) and which has been variously referred to as vitamins or auximones and which is supposed to function somewhat as do the vitamins.

It is evident from the foregoing data that the bacterial stimulant may be organic or inorganic or there may be several different constituents within the soil which function as stimulants to Azotobacter. In order to learn more concerning the nature of this product soils were fractionated and their effects studied when added to Ashby's medium inoculated with nitrogen fixers. The basic medium had the following composition:

K_2HPO_4	0.5 gm.
NaCl	0.2 gm.
$MgSO_4$	0.2 gm.
Distilled water	1,000 cc.

This was made neutral to phenolphthalein with NaOH, and then 0.5 gm. $CaCO_3$ was added to each 100 cc. of the medium. The soil extract medium contained the same constituents as the Ashby's medium, except that the distilled water was replaced by a soil extract prepared by extracting 100 gm. of soil with 1,000 cc. of distilled water. The "evaporated water soil extract" was prepared by evaporating an equivalent quantity of the soil extract to dryness and then adding it to the basic medium. The "soil extract ash medium" was prepared by evaporating the soil extract to dryness, igniting it at a dull red heat, and then adding it to the basic medium. The "alcoholic extract medium" was prepared by extracting 100 gm. of soil with 95 per cent alcohol, evaporating it to dryness, and adding the residue to the basic medium. Baker's "CP" chemicals were used throughout the experiment, and in every case sterile blanks were incubated alongside the inoculated solutions. The *Azotobacter chroococcum* culture used was isolated from the College Farm soil. The inoculated media were incubated at from 28° to 30°C. for 4 weeks in 500-cc. Erlenmeyer flasks containing 100 cc. of the specific medium. The average results for three or more determinations are given in table 3.

TABLE 3

Milligrams of nitrogen fixed in 100 cc. Ashby's and modified Ashby's media by Azotobacter chroococcum

ORGANISM	ASHBY'S MEDIUM	SOIL USED FOR EXTRACT*	ASHBY'S MEDIUM + SOIL EXTRACT	ASHBY'S MEDIUM + EVAPORATED SOIL EXTRACT	ASHBY'S MEDIUM + ASHED SOIL EXTRACT	ASHBY'S MEDIUM + ALCOHOLIC SOIL EXTRACT
	mgm. N		*mgm.* N	*mgm.* N	*mgm.* N	*mgm.* N
Azotobacter chroococcum	3.6±0.1	N	3.7±0.0	3.7±0.0	3.7±0.1	1.9±0.0
	3.6	2	9.5±0.4	3.8±0.2	3.9±0.0	2.4±0.1
	3.6	71	7.1±0.0	6.9±0.3	5.9±0.0	2.1±0.1
	3.6	81	7.4±0.1	7.0±0.0	6.8±0.3	1.7±0.0
	3.6	93	4.0±0.2	4.0±0.2	4.1±0.1	2.0±0.1

* N = Nephi soil; 2 = College farm soil; 71 = College farm soil to which has been added 0.66 per cent of NaCl, Na_2SO_4, and Na_2CO_3; 81 = Corinne alkali soil; 93 = Richland Acres soil.

It is evident that there occurs in these soils a substance or substances soluble in water and capable of stimulating nitrogen fixation when added to Ashby's basic medium. The potency of the stimulant varies with the specific soil from which it was obtained and the microörganism on which it acts. It is most efficient in the case of *Azotobacter chroococcum* when obtained from the College Farm soil and least when obtained from the Richland Acres soil.

The stimulant is partly inactivated by evaporating the soil extract to dryness but is not entirely inactivated by heating to a dull redness. It is insoluble in alcohol or is inactivated by 95 per cent alcohol, since when the alcoholic extract is added to Ashby's basic medium nitrogen fixation is decreased. Consequently, the stimulant is probably due in a measure to inorganic constituents of the soil. The first to be tested was iron, and the average results for a number of determinations with and without iron are given in table 4.

Fixation is greater in the presence of iron than in its absence, and apparently the optimum concentration is about 50 p.p.m. Fixation with a concentration of 10 and 50 p.p.m. of iron is approximately the same, indicating that although the organisms require iron the quantity required is not great; however, they are not injured by considerable quantities. The low fixation reported for these cultures is due to the short incubation period, for it was found that when the medium containing 50 p.p.m. was incubated for longer periods the gains in nitrogen were: 5 weeks 3.3 ±0.1 mgm. nitrogen; 6 weeks 5.4 ±0.2 mgm. nitrogen; and 7 weeks 4.5 ±1 mgm. nitrogen. Consequently, a loss of nitrogen did not occur until after 6 weeks.

TABLE 4

Milligrams of nitrogen fixed by Azotobacter chroococcum *in 100 cc. Ashby's medium at 28° to 30°C. during three weeks with and without iron salts*

Fe	No	1 p.p.m.	5 p.p.m.	10 p.p.m.	50 p.p.m.	100 p.p.m.
Nitrogen fixed (mgm.)	2.0±0.0	2.1±0.2	2.6±0.3	2.9±0.2	3.0±0.1	2.0±0.0

TABLE 5

Milligrams of nitrogen fixed in 100 cc. Ashby's medium with and without manganese during various incubation periods

INCUBATION PERIOD	MGM. OF NITROGEN FIXED		
	Without manganese	With 40 p.p.m manganese	Gains due to manganese
weeks			
4	2.0±0.0	2.3±0.0	0.3
5	2.0±0.0	2.5±0.0	0.5
6	2.2±0.1	2.8±0.0	0.7
7	2.5±0.0	3.5±0.1	1.0
8	2.9±0.2	3.6±0.1	1.3
9	2.8±0.0	3.8±0.1	1.0
10	3.0±0.2	5.0±0.1	2.0
11	2.9±0.3	5.1±0.3	2.2
12	3.0±0.1	5.0±0.2	2.0

Copper and zinc were each tested separately on *Azotobacter chroococcum* in concentrations from 1 to 250 p.p.m. Neither stimulated nitrogen fixation in any of the concentrations used and both manifested marked toxicity at low concentrations. However, results have been reported elsewhere (7) that indicate a stimulation for these elements when used in soil.

Salts of manganese were tested under similar conditions. The results are given in table 5.

There is unmistakable evidence that manganese, both in solution and in soil, increases nitrogen fixation. In solution with *Azotobacter chroococcum* the stimulation varies with the duration of the incubation period. On short incubation periods it is slight, probably because the traces of manganese occurring in the chemicals used to make up the medium are sufficient to meet the needs of the microörganisms. The consistency of the gains lends support to the idea that manganese is an essential nutrient and not just a stimulant. Manganese has proved equally efficient, as has iron, in the use as an Azotobacter stimulant in all of our work. It has been shown elsewhere (11) that stimulation occurs when manganese is added to the soil, and Olaru (22) considers it likely that the increased yield obtained after the application of manganese compounds to a soil is due to its accelerating the action of the nitrogen-fixing microörganisms. He found that manganese in the right concentration has a favorable influence upon *Azotobacter chroococcum* (23).

TABLE 6

Milligrams of nitrogen fixed by Azotobacter chroococcum *in 100 cc. Ashby's medium in the presence of varying quantities of iron and manganese*

TREATMENT	MGM. OF NITROGEN FIXED
Ashby's medium	3.0±0.1
Ashby's medium + 40 p.p.m. Fe	4.0±0.1
Ashby's medium + 10 p.p.m. Mn	4.0±0.0
Ashby's medium + 10 p.p.m. Mn + 40 p.p.m. Fe.	8.4±0.2
Ashby's medium + 20 p.p.m. Mn + 40 p.p.m. Fe.	8.3±0.1
Ashby's medium + 30 p.p.m. Mn + 40 p.p.m. Fe.	8.7±0.1

TABLE 7

Milligrams of nitrogen fixed in the presence and absence of sodium iodide

TREATMENT	MGM. OF NITROGEN FIXED
No iodine.	3.0±0.2
1 p.p.m. iodine.	3.3±0.2
5 p.p.m. iodine.	3.9±0.0
10 p.p.m. iodine.	4.6±0.2
50 p.p.m. iodine.	4.6±0.2
100 p.p.m. iodine.	5.0±0.1

When manganese and iron are used jointly in Ashby's medium there is an increase in nitrogen fixation over that occurring in the presence of either manganese or iron (table 6).

The stimulation exerted by manganese and iron when used together is much greater than the summation of the two individual stimulations. These results make it evident that no medium for the culturing of *Azotobacter chroococcum* can be considered complete without small quantities of both iron and manganese.

Strong evidence has been produced elsewhere (12) that small quantities of iodine are essential for the multiplication of yeast and it is possible that the same may be the case with *Azotobacter chroococcum*. Or in the event that iodine is not essential it may greatly accelerate growth and metabolism.

The average results obtained with *Azotobacter chroococcum* when grown in the presence and absence of iodine are given in table 7.

TABLE 8

Milligrams of nitrogen fixed in 100 cc. Ashby's medium with and without iron and iodine

TREATMENT	MGM. NITROGEN FIXED
Ashby's medium	2.9±0.1
Ashby's medium + 40 p.p.m. Fe	4.0±0.0
Ashby's medium + 10 p.p.m. I	4.6±0.2
Ashby's medium + 10 p.p.m. I + 40 p.p.m.Fe	6.9±0.1

TABLE 9

Milligrams of nitrogen fixed in 100 cc. Ashby's medium with and without manganese, iron and iodine both in the presence and absence of rapid aeration

TREATMENT	MGM. NITROGEN FIXED
Ashby's medium aerated	4.2±0.1
Ashby's medium not aerated	4.3±0.1
Ashby's medium + 40 p.p.m. Mn aerated	4.9±0.1
Ashby's medium + 40 p.p.m. Mn not aerated	4.8±0.0
Ashby's medium + 10 p.p.m. I aerated	4.7±0.0
Ashby's medium + 10 p.p.m. I not aerated	5.8±0.1
Ashby's medium + 40 p.p.m. Fe aerated	7.1±0.1
Ashby's medium + 40 p.p.m. Fe not aerated	7.7±0.1
Ashby's medium + 40 p.p.m. Fe + 40 p.p.m. Mn aerated	6.6±0.0
Ashby's medium + 40 p.p.m. Fe + 40 p.p.m. Mn not aerated	7.4±0.1

Iodine increases the nitrogen fixed by *Azotobacter chroococcum*. The fixation gradually increases as the iodine added increases. At a concentration of 100 p.p.m. of iodine the fixation is increased 70 per cent over that which occurs in the absence of iodine.

When iron and iodine are used jointly in Ashby's medium the increase in nitrogen fixation is a summation of the effects produced when each is used singly (table 8).

Söhngen (24) considered that colloidal substances act by increasing the availability of the oxygen. If this be true increased fixation should result from the greater aeration; consequently, in these experiments *Azotobacter* *chroococcum* has been grown in highly aerated flasks. They were cultured in 500-cc. Erlenmeyer flasks containing the various media. The flasks were fitted with two-hole rubber stoppers. One hole carried a tube bent at 90°, the end of which was drawn to a fine point and dipped into the cultural fluid; the other contained a tube reaching just through the stopper and connecting on the other end with a pump so that sterilized air could be drawn through the solution. The flasks were covered with tar and immersed in a water bath held at 28° to 30°C. by means of an electrical heating device. The non-aerated flasks were stoppered with cotton and incubated alongside the aerated flasks.

The data in table 9 in no way support the theory that iron, manganese, and iodine stimulate Azotobacter by increasing the available oxygen. In no case was there an increased fixation where the flasks were actively aerated; in some cases there was a decrease in fixation due to aeration. It would, therefore seem more reasonable to conclude that manganese, iron, and iodine are elements used in the growth and metabolism of the *Azotobacter chroococcum*.

TABLE 10

Milligrams of nitrogen fixed in Ashby's medium in the presence of varying amounts of aluminum, bromine, copper, and zinc

MEDIUM	MGM. OF NITROGEN FIXED IN PRESENCE OF VARYING QUANTITIES OF				
	$Na_2B_4O_7$	$AlCl_3$	$CuSO_4$	KBr	$ZnCl_2$
Ashby's medium	1.9±0.1	2.5±0.0	4.0±0.1	2.2±0.1	3.2±0.3
1 p.p.m. of element	1.8±0.0	1.4±0.0	0.0	2.5±0.2	2.7±0.0
5 p.p.m. of element	1.8±0.1	1.1±0.0	0.0	2.5±0.1	1.3±0.0
10 p.p.m. of element	1.9±0.1	0.8±0.0	0.0	2.4±0.1	1.1±0.1
50 p.p.m. of element	1.6±0.1	0.6±0.2	0.0	2.5±0.0	0.0
100 p.p.m. of element	1.4±0.1	0.1±0.1	0.0	2.8±0.2	0.0

The influence of boron, aluminum, bromine, and copper was tested on *Azotobacter chroococcum;* the results are given in table 10.

Individually treated sets were run separately; hence, the total fixations in the boron, copper, zinc, etc. sets are not comparable with one another. The results obtained with the varying quantities of each element, however, are directly comparable, since time, temperature, and all other factors, except the quantities of the added element, were held constant. With the possible exception of bromine, none of the elements studied stimulates *Azotobacter chroococcum.* Aluminum and copper both become toxic at 1 p.p.m., the copper being toxic enough to stop all nitrogen fixation at this concentration and the aluminum sufficiently toxic to reduce the fixation about one-half. Zinc becomes toxic at 5 p.p.m. and boron at 50 p.p.m.

Arsenic occurs in some virgin soils and in many cultivated soils (2). It has been shown elsewhere that arsenic greatly increases nitrogen fixation in some soils, but that in liquid media it becomes toxic at extremely low concentrations

(7); consequently, it is not necessary to consider arsenic further here. However, titanium, selenium, and tellurium may all occur in soil and it is possible that they may influence nitrogen fixation by *Azotobacter chroococcum*; consequently, tests were made in the presence of varying quantities of each of these elements. Inasmuch as copper and zinc were used in the preceding tests in comparatively large quantities and may occur in soil in much smaller quantities they were tested in lower concentrations, the results being given in table 11 along with those for titanium, selenium, and tellurium.

TABLE 11

Milligrams of nitrogen fixed by Azotobacter chroococcum *in 100 cc. Ashby's medium containing varying quantities of copper, zinc, titanium, selenium, and tellurium*

	PARTS PER MILLION OF ELEMENT							
	None	0.0001	0.001	0.01	0.1	1.0	10	100
Titanium	6.6±0.2	6.5±0.1	6.3±0.2	6.8±0.3	7.3±0.1	7.0±0.1	6.9±0.3	6.4±0.2
Selenium	6.4±0.2	6.4±0.3	6.3±0.2	5.6±0.3	6.0±0.4	5.4±0.1	5.5±0.1	0.1±0.2
Tellurium	6.4±0.2	6.3±0.1	6.4±0.1	6.3±0.1	6.1±0.1	6.1±0.1	6.1±0.2	6.4±0.2
Zinc	6.3±0.4	6.1±0.2	6.9±0.2	5.5±0.2	4.9±0.1	5.6±0.1	4.7±0.1	0.0±0.1
Copper	6.3±0.2	6.3±0.2	5.5±0.4	5.2±0.4	4.9±0.3	0.6±0.1	0.3±0.0	0.0±0.1

TABLE 12

Milligrams of nitrogen fixed in 100 cc. of Ashby's medium in the presence and absence of various extracts of wheat middlings and alfalfa

TREATMENT	MGM. OF NITROGEN FIXED
Ashby's medium	3.1±0.1
Ashby's medium + water extract of middlings	3.5±0.1
Ashby's medium + water extract of alfalfa	8.3±0.2
Ashby's medium + alcohol extract of middlings	3.1±0.2
Ashby's medium + alcohol extract of alfalfa	4.1±0.2
Ashby's medium + ether extract of middlings	3.1±0.1
Ashby's medium + ether extract of alfalfa	3.8±0.1

There is no evidence in this work that titanium, selenium, tellurium, zinc, or copper are essential for the growth and metabolism of *Azotobacter chroococcum* or even that minute quantities act as stimulants. Copper becomes toxic at 0.001 p.p.m. and practically stops fixation at 1 p.p.m. Zinc becomes toxic at 0.01 p.p.m. and completely stops fixation at 100 p.p.m. Selenium, at a concentration of 100 p.p.m., was sufficiently toxic to stop fixation, but this concentration of titanium and tellurium was without preceptible effect upon *Azotobacter chroococcum.*

In the early part of this work it was shown that the bacterial stimulant contained in soil is soluble in water and loses part of its efficiency on heating. Consequently, it was deemed advisable to study the extractives from plants and plant products. This was done by extracting 200-gm. portions of sterilized wheat middlings and alfalfa with water, alcohol, and ether. Fifty cubic centimeter-portions of each of the extracts were evaporated to dryness in 500-cc. Erlenmeyer flasks; to each flask was added 100 cc. of standard Ashby's medium, after which they were autoclaved, inoculated with a stock culture of *Azotobacter chroococcum*, and incubated for 6 weeks. The total nitrogen was then determined by the Kjeldahl method. Sterile blanks were run in all cases.

The water, alcohol, and ether extracts of middlings were all without effect upon the nitrogen-fixing powers of *Azotobacter chroococcum* when added to Ashby's medium. This is also true of the ether extract of alfalfa, thus excluding the possibility of the stimulant being a fat-like substance. Possibly the alcohol extract of the alfalfa has a slight stimulating effect. The water extract produces a most marked acceleration of nitrogen fixation by *Azotobacter chroococcum*. This latter fact lends support to the theory advanced in this paper that the major stimulation resulting from the addition of soil or plant extract to inorganic synthetic media, such as that of Ashby's, is due primarily to the addition of certain chemical elements, such as iron, manganese, or iodine, which are used in small quantities by *Azotobacter chroococcum* in its growth and metabolism.

TABLE 13

Milligrams of nitrogen fixed in 100 cc. of various cultural media by Azotobacter chroococcum *during 5 weeks' incubation at 28° to 30°C.*

MEDIUM NO.	MEDIUM	MGM. OF NITROGEN FIXED
4	Ashby's basal solution	3.0
130	Beijernick's phosphate glucose salt solution	0.4
133	Löhnis' basal glucose salt solution	0.9
157	Krzemeniewski's glucose salt solution	2.4
163	Harvey's citric acid glucose solution	0.2
170	Jones' modified Ashby's mannitol solution	0.2
177	Pariewitsch's tartaric acid salt solution	0.0
	Soil extract salt solution	8.2
	Basal solution, iron, iodine, and manganese	8.7

Consequently, the medium used for the culturing of *Azotobacter chroococcum* should contain these elements, and the formula which has been found to give maximum fixation is as follows:

Monopotassium phosphate	.02 per cent
Magnesium sulfate	.02 per cent
Sodium chloride	.02 per cent
Calcium sulfate	.01 per cent
Iron as ferrous sulfate	50 p.p.m.
Iodine as sodium iodide	40 p.p.m.
Manganese as mangananous carbonate	40 p.p.m.
Mannitol	1.5 per cent
Distilled water	1,000 cc.

The monopotassium phosphate was dissolved separately and made just alkaline to phenolphthalein by the addition of 0.1 N sodium hydroxide. This solution was distributed in 100-cc. portions into 500-cc. Erlenmeyer flasks. Each flask then received 0.5 gm. of calcium carbonate, after which it was autoclaved at 130°C. for 15 minutes.

This medium has been checked against other media which have been proposed by various workers for the culturing of the nitrogen-fixing microörganisms. The media have been prepared according to the formulae given by Levine and Schoenlein (18). Average results for the different media are given in table 13.

In interpreting these results it must be borne in mind that all media were made of chemicals of the highest purity and of a good grade of distilled water. If low grade chemicals or tap water are used, much greater fixation may be observed; apparently, this is why many workers have obtained good results with these media. However, a synthetic cultural medium should contain known constituents, and this has been the goal in this work. With this new synthetic medium *Azotobacter chroococcum's* nitrogen fixation is just as great as where it is grown in soil extract and nearly three times as great as when grown in Ashby's basal solution. A number of the media gave little or no growth with *Azotobacter chroococcum*; with the new media, however, a luxuriant growth was obtained in all cases.

SUMMARY

An extensive study has been made of the factors governing nitrogen fixation in laboratory media and it was found that the nitrogen fixed in soil and in soil extract media by *Azotobacter chroococcum* is considerably greater than that fixed in the synthetic media used at the present time for the culturing of this microörganism. If one uses soil extract medium and Ashby's medium for the evaluation of the number of nitrogen-fixing microörganisms in soil the results vary with the soil from which the extract is obtained, but it is usually considerably higher where soil extract medium is used than where Ashby's medium is used. The quantities of nitrogen fixed in a medium vary within comparatively narrow limits with the number of organisms inoculated into the medium. One or two organisms may not even survive, and when they do survive or where the numbers added are small, there is a long lag period; consequently the Hiltner and Störmer method for the determination of numbers of bacteria in soils must of necessity give low results.

Boron, aluminum, copper, bromine, zinc, titanium, selenium, and tellurium are non-essential to Azotobacter, and even when used in small quantities in liquid media fail to stimulate *Azotobacter chroococcum*. Copper and zinc were found to be toxic when present in minute quantities.

Manganese, iron, and iodine greatly stimulate nitrogen fixation when added to liquid cultural media. No evidence was obtained that they act by adsorption or that they render oxygen more available. The results point to the conclusion that they are essential elements used by this microörganism in its growth or metabolism. No evidence was obtained that *Azotobacter chroococcum* requires certain organic constituents similar to the vitamins or bioses in their growth. A basal medium is suggested which contains manganese, iron, and iodine and in which *Azotobacter chroococcum* grows profusely and fixed, just as large a quantity of nitrogen as it does in soil extract media; the medium also has the advantage of being of known composition.

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INTERRELATIONSHIPS OF SOIL AND FOREST COVER ON STAR ISLAND, MINNESOTA

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Star Island lies in Cass Lake in northern Minnesota, forming part of a large sandy outwash plain through which the Mississippi flows, and has an area of about 1,200 acres, about a fifth of this being occupied by a small lake (fig. 1). The island lies well within the original evergreen forest and has been protected from destructive fires by the surrounding lake and from lumberman and farmer by the inclusion of most of it within the Chippewa National Forest, the remainder being used for summer residences. The surface varies in general from 6 to 46 feet above the level of the water in Cass Lake, with the greater part, including all the sites at which the soil was examined, more than 15 feet above the water. A detailed topographic map of the island, showing contour lines at 5-foot intervals, is available (3).

The principal forest types on the parts well above the surface of the surrounding lake are, in order of extent, Norway pine, maple-basswood, white pine, and jack pine (1, p. 893–894). There are two areas of white pine, the larger on the east side and the smaller on the west, and four of maple-basswood, three of these being on the southwest, southeast, and northeast points. Jack pine is limited to two small areas, both west of Lake Helen, the smaller being occupied almost entirely by jack pines, whereas on the other Norway pines are almost as numerous. The character of the forest cover on four of the areas, representative of all those sampled, is summarized in table 1 and views of three of the forest types are shown in plate 1. All four types occur on deep sand, popularly known as "jack pine sand," ordinarily occupied by the jack and Norway pine types and justly regarded as one of the least productive soils of the region. In a reconnoissance soil survey of the southeastern townships of Beitrami County, within which lies all of Star Island except some of the most southerly parts, only three soil types were mapped on the island—Cass Lake fine sand, which covers most of the island; beach sand on the lowest lying portions, and peat, which occupies the numerous depressions.

The presence upon such a droughty soil of areas of maple-basswood forest, a type usually regarded as an indicator of very productive soil, suggested that a detailed study might reveal local soil variations that would explain the unusual phenomenon. Rosendahl and Butters, describing the original evergreen forest of Minnesota, stated that the hard maple and basswood were found "in rich woods" or "on rich soil" (4, p. 264, 283) and the white pine "chiefly on clay soils" (4, p. 42), whereas the Norway pine was "chiefly on sandy soils, avoiding calcareous areas" (4, p. 44), and the jack pine occupied "sterile acid soils, often forming almost pure stands on sandy outwash plains" and "absent everywhere from calcareous areas" (4, p. 45). The occurrence of maple-basswood forest in northern Minnesota they regard as limited to "situations that are relatively free from late spring frosts, adjacent to large lakes, or on ridges where there is free air drainage" (4, p. 78).

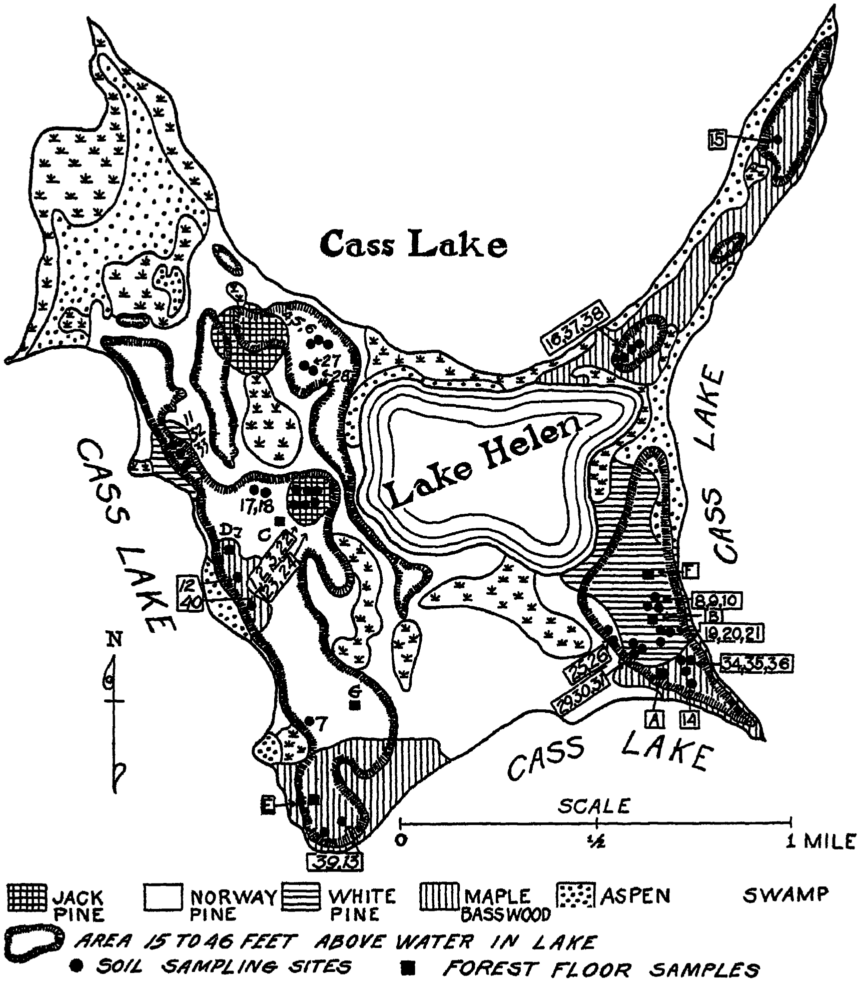

FIG. 1. MAP OF STAR ISLAND SHOWING FOREST COVER AND SAMPLING SITES

The soil profile was studied in 19 pits dug to a depth of 6 feet and the subsoil below was explored with an auger through an additional 5 or 6 feet in order to determine the texture and the depth at which carbonates are first encountered. Samples for laboratory studies were collected by the use of an auger from 21 additional sites. In figure 1 the sites are shown by numbers 1 to 40.

SOIL PROFILE

Underlying the forest floor is the A horizon, a structureless loamy fine or medium sand, varying in thickness from about 1 inch on the jack pine area to a maximum of 5 inches on a maple-basswood area. The bleached layer, A_2, was distinct at all the sites on the jack and Norway pine areas except one, but scarcely distinguishable at most places on the maple-basswood and white pine areas, where the surface soil carries more organic matter, which masks the gray color.

TABLE 1

Number, age, and size of dominant trees in the different forest types, as described by Kittredge (2)

	FOREST TYPES			
	Jack pine	Norway pine	White pine	Maple-basswood
Number of large trees per acre	260	200	100	492
Age, *years*	100	200	250	60
Average height, *feet*	86	100	115	57
Maximum height, *feet*	90	110	120	70
Average diameter, *inches*	11	17	25	7
Maximum diameter, *inches*	14	22	34	16

The B horizon, which consists of structureless loamy fine or medium sand with occasional small non-calcareous pebbles, is from 6 to 11 inches thick, and ranges in color from grayish yellow to grayish brown, in some places with iron stains; although the sand particles are not cemented together the horizon is compact and in the vertical walls of the pits showed no tendency to cave.

The C horizon consists of fine to medium or coarse sand and is not at all sharply separated from the B; in some places small non-calcareous pebbles are distributed throughout the whole of it, in other places only through the lower part and in still others they are entirely absent (fig. 2). In color the horizon is grayish yellow, mottled with brown; with increasing depth the brown becomes more pronounced and on the lighter background produces a marbled effect. Thin bands of sand cemented with iron oxide, from one-eighth to one-quarter inch thick and lying more or less horizontally, are usually encountered below the third foot, in some places even in the lower part of the second foot but in others not above the fifth foot. These are more numerous at the lower levels and in some places are so close together that a section 6 inches or more thick may appear to form a continuous loosely cemented layer.

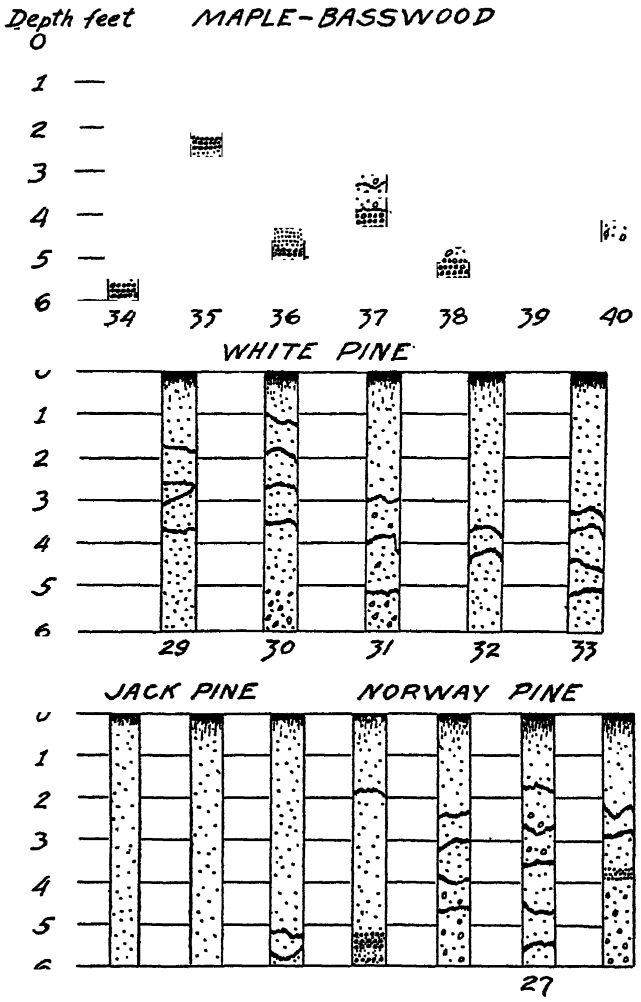

Fig. 2. Profiles under Different Forest Types

The numbers refer to the sites shown in figure 1. Layers of stratified coarse sand and gravel are indicated as in 34 and 35, pebbles as in 39 and 40, bands of iron cemented sand as in 39 and 29.

TEXTURE

As deep as explored the texture was coarse, as shown by the moisture equivalents reported in table 2. Below the portion of the surface foot affected by organic matter they are similar under the different forest types, all low, and with no regular increase or decrease with increasing depth. The higher values for sections below 2 feet, as shown in the last column of the table, are mostly those of samples containing more or less iron-cemented sand. In the surface

3-inch layer the moisture equivalent is much the highest on the maple-basswood areas, which is to be attributed to the higher organic matter content. At all 40 cites, to a depth of 3 feet, the texture is very similar, a loamy fine to medium sand, with an occasional pebble in some of the profiles. Below the third foot there is considerable variation; in 7 of the 19 pits coarser sand and gravel were found in one or more stratified layers, 2 to 6 inches thick, with pebbles in some of the gravel layers. The stratified coarse material is not limited to, or definitely associated with, any one of the forest types but appears most common on the maple-basswood areas; it was found in five of the seven pits under this forest type and in two of the four on the Norway pine areas (fig. 2). The greatest uniformity in texture throughout the depth explored was found on the jack pine area where the soil was a fine to medium sand, free of gravel and pebbles. The knoll on which this area is located, probably of aeolian origin, is small. On the small white pine area to the west, where two pits were dug, Nos. 32 and 33, the subsoil was free of gravel and pebbles and almost as uniform as on the jack pine knoll.

TABLE 2

Moisture equivalents of soil under the different forest types on Star Island

Averages of values determined at 40 sites

DEPTH	JACK PINE, 6 SITES	NORWAY PINE, 10 SITES	WHITE PINE, 12 SITES	MAPLE-BASSWOOD, 12 SITES	RANGE, ALL 40 SITES
1–3 inches	5.8	8.1	8.2	13.9	4.3–16.8
4–6 inches	3.9	4.6	5.0	5 6	3.5– 7.0
7–12 inches	3.5	3.9	4.1	4.0	1.9– 6.7
Second foot	2.5	2.4	2.6	2.8	1.5– 4.4
Third foot	2.0	2.2	2.0	2.4	1.1– 3.4
Fourth foot	1.9	2.1	2.2	2.3	1.2– 4.8
Fifth foot	1.9	2.7	2.4	2.8	1.3– 6.9
Sixth foot	3.0	3.2	2.5	2.5	1.3– 6.1
Seventh foot	2.6	2.7	2.6	2.6	1.8– 3.9
Eighth foot	...	2.0	2.6	...	1.8– 3.1
Ninth foot	...	3.2	2.1	...	1.9– 3.9
Tenth foot	...	4.1	2.0	...	1.9– 5.7
Eleventh foot	...	2.7	2.0	...	1.9– 2.7

MECHANICAL COMPOSITION

In table 3 are reported mechanical analyses of eight sections from each of three profiles, one in the jack pine area and the others in two maple-basswood areas. With the 1–3 and 4–6 inch sections the organic matter was removed with hydrogen peroxide before the separation was begun.

In the uniform profile from the jack pine area fine sand is the main constituent, varying only between 65 and 72 per cent, while fine and medium sand together form between 78 and 91 per cent of the whole. Coarse sand amounts to less than 4 per cent and gravel is absent. In the profiles from the maple-basswood areas there is less fine sand in both, some gravel, and in the one considerable silt.

TABLE 3

Mechanical composition of soil in three representative profiles, showing the preponderance of sands

DEPTH	COARSE GRAVEL	FINE GRAVEL	COARSE SAND	MEDIUM SAND	FINE SAND	VERY FINE SAND	SILT AND CLAY
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1. In jack pine forest. Site 24							
1–3 inches	..	..	1	12	66	11	10
4–6 inches	..	..	1	11	71	9	8
7–12 inches	..	..	1	12	71	10	6
Second foot	..	..	1	14	72	9	4
Third foot	..	..	3	21	67	7	2
Fourth foot	..	..	3	26	65	4	2
Fifth foot	..	..	2	24	66	6	2
Sixth foot	..	..	2	18	71	6	3
2. In maple-basswood forest. Site 34							
1–3 inches	1	4	12	23	29	5	26
4–6 inches	2	6	15	27	32	4	14
7–12 foot	6	6	14	26	36	3	9
Second foot	2	6	12	24	51	2	3
Third foot	2	5	12	32	44	1	4
Fourth foot	3	5	17	50	22	1	2
Fifth foot	..	4	22	57	13	1	3
Sixth foot	22	21	23	22	8	1	3
3. In maple-basswood forest. Site 39							
1–3 inches	..	3	8	20	38	7	24
4–6 inches	..	3	7	22	43	8	17
7–12 inches	..	2	5	20	51	11	11
Second foot	..	8	14	29	41	4	4
Third foot	1	5	10	24	39	11	10
Fourth foot	..	3	12	33	27	11	14
Fifth foot	..	1	5	33	21	17	23
Sixth foot	1	3	13	51	18	4	10

NITROGEN

The differences in organic matter content are indicated by the nitrogen percentages reported in table 4, the averages from the 19 pits. It will be seen that there are marked differences in the surface 3-inch layer, in general the nitrogen being much the highest on the maple-basswood areas with only a third to half as much under the white and Norway pines and still less under the jack pines. In the second 3-inch layer the nitrogen is much lower, being alike on the three pine types, whereas in about half the samples from the maple-basswood areas it is higher than under the pines. Below the surface 6 inches the nitrogen is alike under all the forest types, being low at all depths.

ACIDITY

Table 5 shows the range in pH values determined electrometrically by the use of a bubbling hydrogen electrode and constant mechanical shaker. Under all four forest types the soil profiles are very much alike in the second, third, and fourth feet but in the sixth foot the acidity is in general lower under the maple-basswood. The profile at site 39, in the maple-basswood forest on the southwest point of the island, alone formed an exception, it being as acid as the profiles under the jack pine and Norway pine.

TABLE 4

Nitrogen content of forest floor and of underlying surface foot of soil

	JACK PINE	NORWAY PINE	WHITE PINE	MAPLE-BASSWOOD
	per cent	*per cent*	*per cent*	*per cent*
Forest floor	0.91	1.07	1.15	1.71
1–3 inches	0.04	0.08	0.08	0.21
4–6 inches	0.02	0.03	0.03	0.04
7–12 inches	0.01	0.02	0.02	0.01

TABLE 5

Range of pH values in soil of Star Island under different forest types

	JACK PINE	NORWAY PINE	WHITE PINE	MAPLE-BASSWOOD
	pH	*pH*	*pH*	*pH*
Forest floor	4.4–4.9	4.7–5.3	5.6–6.1	6.3–7.5
1–3 inches	4.7–5.1	5.0–5.2	5.2–5.5	5.2–6.6
4–6 inches	5.1–5.4	5.3–5.5	5.2–5.9	5.0–6.3
7–12 inches	5.5–5.7	5.4–5.8	5.4–5.8	5.1–6.9
Second foot	6.0–6.1	5.4–6.1	5.4–6.3	5.6–6.5
Third foot	6.2–6.5	6.0–6.3	5.8–6.3	5.9–6.7
Fourth foot	6.3–6.4	6.1–6.6	6.0–6.6	5.3–6.6
Fifth foot	6.1–6.6	5.8–6.6	5.9–7.8	5.3–8.0
Sixth foot	5.6–6.3	6.2–7.9	6.2–9.1	6.0–8.4

In all three pits on the jack pine area a decrease in acidity is found on passing from the forest floor to the surface 3-inch section and a similar change occurs in three of the four pits under the Norway pines. In all seven of these profiles a decrease in acidity is found in passing from the 1–3 inch to the 4–6 inch section, a further decrease from the latter to the 7–12 inch section, again a decrease from this to the second and third feet.

On the maple-basswood areas all seven of the profiles show a marked increase in acidity on passing from the forest floor to the surface 3-inch section, and in five there is a further increase from this to the second 3-inch section. From the latter to the 7–12 inch section there is an increase in three cases and a decrease in four. From the lower half of the first foot to the fifth or sixth foot there is, in general, little change, but at this depth a sharp decrease is observed.

In the profiles under the white pines a distinct increase in acidity is found on passing from the forest floor to the surface 3-inch section but it is less marked than under the hardwoods; in three of the five pits the second 3-inch section is more acid than the first, but in two the reverse is true; from the 4–6 inch section to the 7–12 inch there is either little change or some decrease in acidity and from the latter to the second foot a marked decrease, which continues to the fourth or fifth foot.

TABLE 6

Depth of calcareous subsoil below surface on Star Island

MAPLE-BASSWOOD		WHITE PINE		NORWAY PINE		JACK PINE	
Site	Depth	Site	Depth	Site	Depth	Site	Depth
	feet		*feet*		*feet*		*feet*
13	4.0	19	4.0	28	6.3	24	8.5
35	4.5	10	5.0	7	6.5	1	7.+
36	4.5	20	5.0	27	6.8	2	7.+
40	4.7	9	5.5	17	7.0	3	7.+
38	4.8	31	5.8	25	7.0	22	11.+
12	5.0	29	6.5	26	8.0	23	11.+
16	5.0	8	7.0	4	7.+	..	.....
34	5.5	30	7.5	5	7.+	..	.....
14	6.0	32	7.5	6	7.+	..	.....
37	6.5	33	9.0	18	8.+	..	.....
39	7.3	21	10.0	..	....	..	.....
15	7.+	11	7.+	..	....	..	.....

From the foregoing it is evident that differences in soil acidity characteristic of the different forest types on the island are most likely to be found either below 4 feet or in the forest floor and the immediate surface of the underlying soil.

DEPTH TO CALCAREOUS SUBSOIL

Table 6 shows where calcareous subsoil was first encountered in 29 profiles, that is where dilute hydrochloric acid caused distinct effervescence, and the depth to which the examination was carried in the 11 others without reaching it. The calcareous material continued downward from the point where first encountered to the full depth of exploration. In general it is considerably farther from the surface under the pines than under the maple-basswood forest, it being only 7.3 feet below the surface in the most acid profile of the latter.

FOREST FLOOR

Although the soil is essentially alike under the different forest types there are marked differences in the forest floor, which as sampled in this study included all three layers—litter, duff, and leafmold. On composites made a year apart determinations were made of volatile matter, nitrogen, and lime (table 7). The lime reported is that extracted from the ash by *aqua regia*. The differences in nitrogen and lime in the forest floor are best shown as computed on the basis of the volatile matter. Lime is very much the highest in the maple-basswood floor and lowest under the Norway pines. Nitrogen also is highest in the maple-basswood floor. The lime in the surface 3 inches of mineral soil, very low in all compared with that in the forest floor, is highest under the maple-basswood and lowest under the Norway pines.

TABLE 7

Composition of forest floor

A collected in August 1925 and B in October 1926

	MAPLE-BASSWOOD		WHITE PINE		NORWAY PINE	
	A	B	A	B	A	B
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1. Forest floor						
Volatile matter in sample	49.62	59.57	53.36	56.89	36.63	53.67
Ash in sample	50.38	40.43	46.64	43.11	63.37	46.33
N in sample	1.79	1.56	1.36	0.88	0.63	0.86
CaO in sample	3.05	2.62	1.93	1.13	0.60	0.93
N in volatile matter	3.61	2.62	2.55	1.55	1.72	1.60
CaO on basis of volatile matter	6.15	5.23	3.51	2.99	1.64	1.73
2. Surface 3 inches of soil						
Volatile matter	5.33	3.66	2.50	1.86	2.33	2.09
Ash in sample	94.67	96.34	97.50	98.14	97.67	97.01
N in sample	0.20	0.12	0.12	0.05	0.05	0.05
CaO in sample	0.38	0.25	0.16	0.16	0.10	0.12
N in volatile matter	3.78	3.28	4.80	2.68	2.15	2.40
CaO on basis of volatile matter	7.17	6.83	6.40	8.68	4.30	5.74

ACIDITY OF FOREST FLOOR COMPARED WITH THAT OF UNDERLYING SOIL

For a comparison of the acidity of the forest floor with that of the underlying 3 inches of mineral soil the pH was determined in the case of 80 pairs of samples, 34 from the maple-basswood areas, 24 from the jack and Norway pine, and 22 from the white pine; the locations are indicated on the map in figure 1. The forest floor was the less acid of the two in all pairs from the maple-basswood areas, and in two-thirds of those from the white pine but in only one-sixth of the pairs from the jack and Norway pine areas (table 8). In general wherever the pH of the forest floor was below 5.1 the underlying soil was the less acid whereas elsewhere it was the more acid.

TABLE 8

Distribution, according to pH, of samples of forest floor and underlying 3 inches of mineral soil

pH	FOREST FLOOR			SURFACE 3 INCHES OF SOIL		
	Jack and Norway pine	White pine	Maple-basswood	Jack and Norway pine	White pine	Maple-basswood
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Under 5.1	75	9	0	50	18	9
5.1–5.5	25	32	9	50	41	23
5.6–6.0	0	36	9	0	36	35
6.1–6.5	0	23	35	0	5	15
Over 6.5	0	0	47	0	0	18

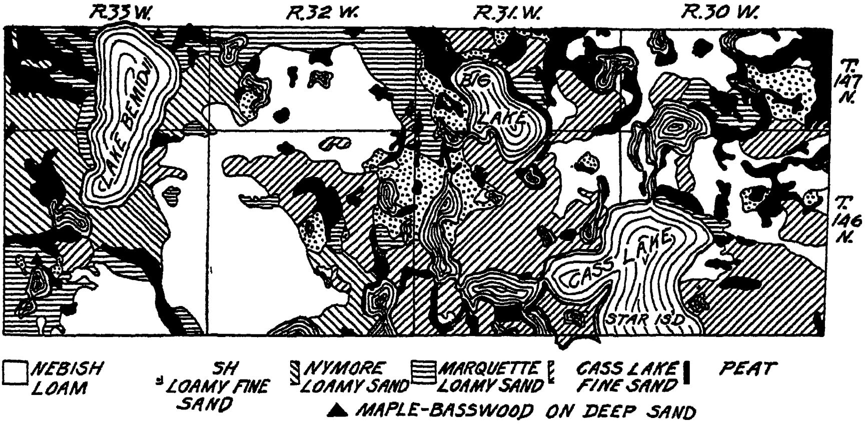

FIG. 3. SOIL MAP OF PART OF BELTRAMI COUNTY INCLUDING STAR ISLAND

Location of two areas of maple-basswood forest on deep sand near the west side is shown

FOREST COVER AS AN INDEX OF SOIL CHARACTER

The exceptional character of the interrelationship of forest cover and soil type on Star Island is well illustrated by the soil map of the southeastern part of Beltrami County, which includes all of Star Island except the southern edge (fig. 3). In this area of 216 square miles six soil types were mapped, viz.: a loam, three deep sands, a type covering the transition between any of these three sands and the loam, and lastly peat. The similarities and differences among the five mineral soil types are illustrated by table 9, giving the moisture equivalent, and the nitrogen content of a representative set of samples from each.

The Nebish loam, developed on the calcareous till plain of the Keewatin glacial drift, has a cover of deciduous trees, chiefly aspen and paper birch, with hard maple and basswood in places; originally it carried a large proportion of white pines. The Cass Lake fine sand and Nymore loamy sand are occupied by jack pines with a small and variable admixture of Norway pine. The Marquette loamy sand in most places has a cover of jack and Norway pines, but here and there are found patches of aspen and paper birch. This soil type, developed upon coarse morainic material, carrying much gravel and stone mixed with the sand, has a better developed profile than the Nymore and Cass Lake types, with a deeper and more retentive horizon B, and within short distances varies greatly in its water retentiveness and natural vegetation

TABLE 9

Texture and nitrogen content of mineral soil types on mainland in vicinity of Star Island illustrated by set of samples from each

DEPTH	MOISTURE EQUIVALENT					NITROGEN				
	Cass Lake fine sand	Nymore loamy sand	Marquette loamy sand	Nebish loamy fine sand	Nebish loam	Cass Lake fine sand	Nymore loamy sand	Marquette loamy sand	Nebish loamy fine sand	Nebish loam
						per cent	*per cent*	*per cent*	*per cent*	*per cent*
1–6 inches	4	5	9	6	13	0.03	0.06	0.07	0.04	0.05
7–12 inches	3	4	4	6	16	0.01	0.02	0.02	0.02	0.03
Second foot	2	4	6	7	20	0.01	0.01	0.01	0.01	0.03
Third foot	2	3	5	12	20	0.01	0.01	0.01	0.01	0.03
Fourth foot	1	2	3	18	22	0.01	0.01	0.01	0.01	0.01

The Nebish loamy fine sand, which has a variable forest cover, most commonly aspen-paper birch but elsewhere jack and Norway pines, is not a uniform soil type but forms the transition from the Nebish loam on the one side to the Cass Lake fine sand or to the Nymore loamy sand or to the Marquette loamy sand on the other. It has developed upon wind deposited or outwash sand, which mantles the till, varying in depth from less than a foot to more than 5 feet. The surface of the sand cover is uneven as is also that of the underlying till, with the result that the thickness of the sand layer and consequently the drouthiness of the soil varies greatly; where the sand is deep, jack and Norway pines are found, but where shallow, a hardwood forest cover occurs, as on the Nebish loam.

From foregoing it will be seen that in the area under consideration jack and Norway pine may well suggest to the soil surveyor the presence of Cass Lake fine sand or Nymore loamy sand, whereas, the aspen-birch or sugar maple-basswood type suggests Nebish loam. When he finds hardwood on a sandy surface he will at once suspect Marquette loamy sand or Nebish loamy fine sand and explore with his auger or spade to decide which. In the course of the survey the forest cover was found so closely correlated with the character of the soil that, in checking the field work, whenever a deciduous cover was found where the soil map showed Cass Lake fine sand or Nymore loamy sand or where jack pines were on land mapped as Nebish loam it was at once suspected that an error in mapping had been made and the soil was reëxamined.

Kittredge, who has made a quantitative study of the correlation between forest types and soil types on some areas of northern Minnesota of which the reconnoissance soil survey had been made some years earlier, concludes that a well defined relationship exists and can be expressed quantitatively in such a way as to enable the use of soil survey maps for the estimation of areas of forest types. In his field study he followed traverse lines which had previously been used by the soil surveyors and mapped the occurrence of the forest types to the nearest one-tenth of a mile. The percentages of each soil type occupied by different forest types provide quantitative expression for the correlation between forest and soil. His data for the areas near Cass Lake, in so far as they deal with the five mineral soil types shown in figure 3, are given in table 10. The occurrence of a forest type on a small proportion of a soil type with which it is not correlated he attributes to the narrow strips which form the transition between contiguous soil types and are not typical of either. On these strips, too narrow to be shown on the map, there is usually a change in the forest type, and soil mapper and forest mapper, working independently, do not place the boundary at the same point.

TABLE 10

Correlation of forest type with soil type by Kittredge, showing percentages of total traverse on each soil type occupied by the various forest types*

SOIL TYPE	FOREST TYPES						TRAVERSE LENGTH
	Jack pine	Norway pine	Aspen	Sugar maple-basswood	Spruce-fir	Swamp forest	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*miles*
Nymore loamy sand	100	..	..	..	..	..	4
Cass Lake fine sand	89	..	7	..	..	4	23
Marquette loamy sand	71	9	10	1	9	..	18
Nebish loamy fine sand	23	1	70	..	..	6	7
Nebish loam	3	..	85	6	..	6	29

* Data from an unpublished study by J. Kittredge.

Two other tracts of maple-basswood forest on deep sand have been found in the area covered by the map shown in figure 3, both in situations having unusual protection from forest fires, but no detailed study has been made of either.

The larger occupies about 60 acres on the northeastern shore of Lake Bemidji and part is now included in the Bemidji State Park. On the greater portion of it the surface is from 25 to 55 feet above the water of the lake. The second, on the northeast shore of Lake Marquette and shown near the southwest corner of the area, occupies less than two acres. On both tracts the growth of the maples and basswoods appears much the same as on Star Island.

Kittredge, who has recently made a study of the vegetation of Star Island, explains the occurrence of the maple-basswood along with the Norway and jack pine forest types, as due to the maple-basswood being the climax upland forest of the region, even on such sandy soils, and believes that it will be found wherever an area has been protected from fires for a sufficient length of time (2). He considers the sandy soil of the island to provide a poor site for maple-basswood forest, an only medium site for white pine, but a good site for Norway pine, basing this conclusion upon the average diameters and heights of the different species compared with their ages (2). If this is so, the presence of mature maple-basswood forest does not necessarily indicate the presence of a water-retentive soil but merely prolonged protection from forest fires, as well perhaps as a situation "relatively free from late spring frosts" (4, p. 78). However, it appears that a maple-basswood forest in which the trees have made a *normally rapid growth* may still be considered an index of productive soil.

SUMMARY AND CONCLUSIONS

On a small sandy island in a northern Minnesota lake four forest types are found in a practically virgin condition, viz: jack pine, Norway pine, white pine, and maple-basswood. In the surrounding region the maple-basswood and white pine types are found on the heavier, more drouth-resistant, productive soils, whereas the Norway pine and jack pine are characteristic of the drouthy, deep sands, the most unproductive mineral soils of the region.

The soil was examined to a depth of 7 to 12 feet at 40 places where the surface is from 15 to 46 feet above the water in the lake and only coarse textured material is found. No differences in the soil were found to account for the presence of the maple-basswood type on the sandy soil. Under all four forest types the soil profile is similar, except that in the surface 6 inches of the maple-basswood areas there is much more organic matter than under the jack and Norway pines and the acidity is in general somewhat lower. In the second, third, and fourth feet there were no characteristic differences in acidity, but at greater depths the acidity was lower on the maple-basswood areas and on these carbonates were usually found nearer the surface, at 4.0 to 7.3 feet.

The forest floor shows more characteristic differences, that on the maple-basswood areas being much less acid, much richer in lime, and somewhat richer in nitrogen than that on the jack and Norway pine areas. Compared with the underlying surface 3 inches of soil the forest floor on the maple-basswood areas is almost everywhere the less acid whereas on the jack and Norway pine areas it is usually the more acid. The marked differences in the character of the forest floor and smaller differences in the soil may be considered the effect of the forest types still occupying the different parts of the island.

In the character of both forest floor and soil the white pine areas occupy a position between the jack and Norway pine on one side and the maple-basswood on the other.

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(3) MISSISSIPPI RIVER COMMISSION 1915 Map of the Mississippi River from Minneapolis, Minn. to Lake Itasca. Charts Nos. 264 and 265.

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PLATE 1

CHARACTER OF FOREST ON STAR ISLAND

FIG. 1. Norway pine on west shore.

FIG. 2. In white pine forest near east shore. Large trees about 250 years old.

FIG. 3. In sugar maple-basswood forest south of area shown in figure 2. Larger trees about 60 years old.

Fig. 1

Fig. 2

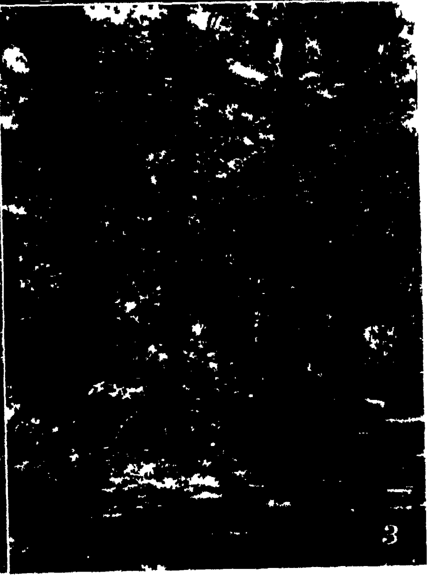

Fig. 3

A SENSITIVE HYDROMETER FOR ESTIMATING TOTAL SOLIDS IN IRRIGATION WATERS AND SOIL EXTRACTS

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By far the most important factor influencing the quality of irrigation waters is the amount of dissolved salts in them. This is usually determined in the laboratory by evaporating a known volume of water and weighing the residue. The measurement of the electrical conductivity also affords a means of estimating total solids in water and this has the advantage that it can be used as a field method. However, the apparatus required is costly and needs careful handling; besides, the results obtained are not always very reliable.

Since dissolved substances raise the density of water proportionately to the amount of solute, it appeared reasonable to suppose that if small differences in density could be measured accurately and by a simple technique, it would afford a method of estimating total solids in irrigation waters and soil extracts. The hydrometer is the simplest of all methods for measuring density of liquids, but it is by no means the most accurate, for, since the water surface does not provide a sharp line of reference, an appreciable error in the readings might be easily made. In addition, though a hydrometer can be made as sensitive as one desires by increasing the volume of the bulb and decreasing the thickness of the stem, an increase in sensitivity makes the hydrometer also sensitive to variations in the density of water, due to changes in temperature; and unless the latter could be controlled to the fraction of a degree the density changes due to dissolved substances might be masked to a large extent by temperature variations. This is especially important for regions showing extremes of climate like the north of India. Here it is quite possible that a sensitive hydrometer giving zero reading in pure water during summer might go entirely off the range in the same water in winter. These difficulties have been successfully overcome in the hydrometers described in this paper.

DESCRIPTION OF HYDROMETERS

Two types of hydrometers were constructed one for laboratory use and the other for field work. These are described in the following.

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[1] It is my pleasant duty to record my indebtedness to Dr. E. McKenzie Taylor, director, Irrigation Research Institute, Punjab, for his helpful discussions of results, and approval of the paper for publication.

Laboratory type

This consists of a glass spindle about 4 cm. in diameter and 12 cm. long to which is attached a thin glass tube 4 mm. in diameter and about 20 cm. long. The bulb of a broken 100-cc. pipette can be used for this purpose and practically no skill in glass blowing is required to make a serviceable instrument. The hydrometer is weighted with mercury or lead shots till it floats in distilled water at a convenient depth. A modification in the hydrometer technique is the arrangement for taking readings. This has already been described in connection with a hydrometer for mechanical analysis of soils.[2] The usual method of inserting a paper scale in the stem is not very accurate, being subject to errors due to parallax. In this case the tip of an ordinary pin attached to a thin brass cap fitting on the top of the hydrometer forms the reference point,

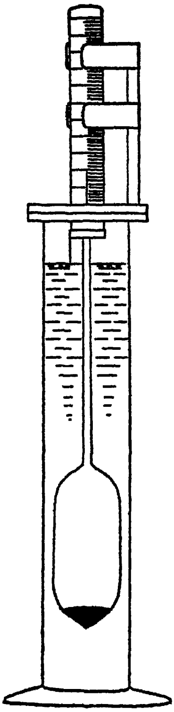

FIG. 1

FIG. 1. DIAGRAM OF THE LABORATORY TYPE OF HYDROMETER

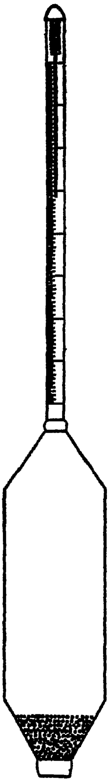

FIG. 2

FIG. 2. DIAGRAM OF THE FIELD TYPE OF HYDROMETER

and the readings are taken against the graduations on a burette tube, held in special clamps (fig. 1). The mouth of the burette tube is closed with a brass cap having a hole in the middle through which the hydrometer stem is passed. The edges of the hole are sharpened to prevent friction that might hinder the free movement of the hydrometer. All that is necessary is to tap the burette tube gently before taking a reading. This brass cap has also a pin attached to it which is required for fixing the position of the burette tube with respect to the water surface. The tube is gradually lowered till the pin point just makes contact with the water surface. The burette tube is held in brass clamps which keep it in position, at the same time allowing it to be pushed up or down. The brass clamps are fixed to an iron rod attached to an iron cap that can cover the top of the cylinder containing the water sample. The iron cap has a hole in the middle to allow the free movement of the burette tube. This simple device enables one to take hydrometer readings to a fraction of a millimeter and does away with the necessity of having a paper scale inside the stem. The same reading device can be used for a number of hydrometers which are calibrated in terms of the graduations on the same burette tube. To avoid parallax the graduations on the burette tube should be all round the circumference as in the Charlotenburg type.

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[2] PURI, A. N. 1932 A new type of hydrometer for the mechanical analysis of soils. *Soil Sci.* 33: 241–248.

Hydrometer for field work

For field work a glass hydrometer is unsuitable on account of the possibility of breakage. In the laboratory, temperature changes can be allowed for by taking readings with distilled water simultaneously, this is not possible in survey work. The chief feature of the field hydrometer is the temperature compensation incorporated in the body of the instrument.

The hydrometer, which is made of brass and is nickle plated, has a spindle 10 inches long and 2.5 inches in diameter. The stem which is screwed to the spindle is 12 inches long and about 0.3 inch in diameter and has 0.05-inch graduations engraved on it. The top of the stem has a hollow screw cap threaded on the inside to take a brass wire about 1.5 mm. thick (fig. 2). For calibration the spindle is weighted with lead shots by unscrewing and screwing the stem till the hydrometer shows zero reading in distilled water at 35°C. The stem is then attached to the spindle more firmly by heating and running some sealing wax in the threads and screwing while hot. The hydrometer is next placed in water at 34°C. and a small piece of brass wire threaded at the tip is screwed on to the cap. The weight of the brass wire is adjusted by cutting and rubbing on a flat file till the hydrometer again reads zero at 34°C. The temperature is then brought to 33°C. and another wire screwed on to the cap after detaching the first. The weight of the wire is again adjusted to make the hydrometer register zero reading at that temperature. Similarly the hydrometer is adjusted with suitable weights of wires to give zero readings at other temperatures down to 15°C., or even below if necessary. The wires appropriate for various temperatures are arranged in a box with the corresponding temperatures written against them. Before making a density determination the temperature of the water is taken and the appropriate wire corresponding to that temperature screwed on. The hydrometer reading is then directly proportional to the concentration of the salts and once the instrument is calibrated it becomes a simple matter to determine total solids in a given sample of water. If the temperature is slightly higher or lower than that corresponding to the temperature correction wire, a positive or negative correction is applied to the hydrometer reading. The temperature should be read with a thermometer reading to 0.2 of a degree.

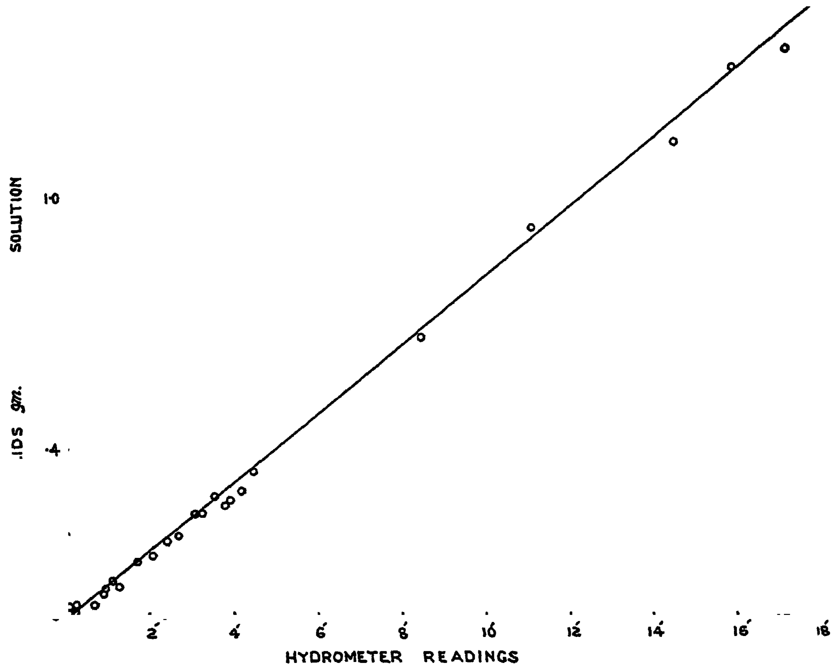

FIG. 3. RELATION BETWEEN HYDROMETER READINGS AND TOTAL SOLIDS IN SOIL EXTRACTS

EXPERIMENTAL

In order to determine whether the excess of density of the solution over that of water is proportional to the concentration of dissolved salts, a number of soil extracts were examined by the laboratory type of hydrometer. The extracts were obtained by shaking soils with water in 1:5 ratio for 24 hours and filtering through Pasteur-Chamberland filters. One hundred cubic centimeters of the filtrate was evaporated to dryness for total solids and the remainder used for density measurement with the hydrometer. The results plotted in fig. 3 show that the relation between hydrometer readings and total solids is practically linear and therefore the hydrometer could be used for measuring the percentage of dissolved material in irrigation waters or soil extracts. It must be admitted that the density of a solution is also dependent on the nature of the dissolved substances, but variations due to this factor are not serious when dealing with natural irrigation waters and soil extracts. In fact all indirect methods like the conductivity and freezing point methods recognize this fact.

The field hydrometer has been found very useful for determining the quality of well waters for irrigation purposes. The hydrometer stem can be painted in three colors showing good, medium and bad waters. With medium waters a few waterings are admissible, provided canal irrigation, though limited, is available. Bad waters can be rejected at once. It is not infrequent to find farmers ruining their fields by irrigating with well waters having total solids much above the safe limit.

This type of hydrometer has also been successfully employed for finding the silt content of canal and river waters especially during floods. This is simply done by taking a reading immediately after stirring the water and one after the hydrometer has reached a steady stage, i.e. when all the silt has settled down.

SUMMARY

The percentage of total solids in soil extracts and irrigation waters can be determined with a reasonable degree of accuracy by hydrometer.

Two sensitive hydrometers have been described for the purpose, one for laboratory use and the other for field work.

STUDIES ON READILY SOLUBLE PHOSPHATE IN SOILS: III.[1] THE EFFECT OF PHOSPHATE TREATMENT

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These investigations were undertaken in order to study the relative effectiveness of different phosphates in increasing the readily soluble phosphate content of typical phosphate deficient soils. Although the ultimate solution to this problem of economically correcting phosphate deficiency has to be obtained by suitable field experiments in which plant response is the measure of effectiveness, chemical studies in the laboratory are desirable to establish the fundamental principles on which such experiments may be based.

It is the purpose of this paper to present data from such preliminary chemical studies which are forerunners to plant nutrition and field experiments on the phosphate problem in this province.

REVIEW OF LITERATURE

The literature dealing with the problems taken up in this paper is quite voluminous; therefore, only a few typical references bearing directly upon the interpretation of the data presented here will be mentioned.

Data from permanent field experiments with different phosphates have shown that the more slowly soluble phosphates, such as some rock phosphates, basic slags, and the like, can give good results under certain circumstances (22, 25, 34). This phenomenon is especially true for slowly growing crops on acid soils and in the case of the application of rock phosphate when it is accompanied by decaying organic matter. It must be borne in mind, however, that the total amounts of phosphorus (P) added to the rock phosphate treated plots, in Hopkins and Roberts' experiments greatly exceed the total amounts of phosphorus (P) given in the superphosphate treated plots.

That conversion and fixation of water-soluble phosphate take place in the soil, is proved by the fact that phosphate accumulation takes place in permanent plots having long continued treatment with superphosphate (3, 8, 19). Also, the amount of water-soluble phosphate in most soils is very low (43).

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[2] Research assistant and assistant professor, respectively. Acknowledgments are due to Prof. R. Harcourt, head of the department, for encouraging and supporting this series of researches; and to Profs. D. R. Hoagland, and P. L. Hibbard, and to Dr. H. D. Chapman, University of California, for valuable advice given to the authors in connection with the problems dealt with in this series of papers. The authors only are responsible for the interpretation of the data and the conclusions drawn.

[3] Published with the permission of the president.

It is supposed that the calcium-ortho-phosphates, in *faintly acid* and *alkaline* soils, become ultimately converted into calcium-hydroxy-apatite, $3\ Ca_3(PO_4)_2 \cdot CaOH)_2$, which is the only calcium phosphate stable in this reaction range (1, 2, 6, 16, 40, 41). The conversion of the ortho-phosphates of calcium into hydroxy-apatite is a very slow process, and there is much evidence for the assumption that fixation of soluble phosphates in soils, as far as it takes place more *immediately*, is due largely to conversion into iron and alumina phosphates. From the examination of a large number of Texas soils, Fraps found a close correlation between the amount of soluble phosphate fixed by these soils and the quantities of iron and alumina soluble in strong acid (15). Gaarder (16) from a very detailed study on the solubility of the phosphates of Fe^{III}, Al, Ca, and Mg found that with an excess of all these bases together almost complete precipitation of the phosphate ion can take place above a pH value of 2.9. Since the content of bases in a soil so largely exceeds the content of phosphorus, the formation of difficultly soluble soil phosphates is easily comprehended (7). Britton (4) found that mono- and di-calcium phosphates are not precipitated from solutions more acid than approximately pH 5.5. In *acid* soils, therefore, these calcium phosphates are, no doubt, converted into iron and aluminum phosphates. The mechanism of this conversion is explained by the fact that colloidal iron and aluminum phosphates can precipitate soluble phosphates (16, 17, 18). There is no doubt that rather difficultly soluble phosphates are formed under such conditions, since acid soils generally are deficient in readily soluble phosphates (38). Regarding the value of the phosphates thus converted in the soil, actually not much is known. It is quite probable, however, that the converted phosphate may, to some extent, be more or less useful in plant nutrition (9, 18, 30). Some fixation of the soluble phosphates in the humus fraction is also likely (35). In soils where organic matter is decaying, rock phosphate becomes more soluble (22). The same is true in soils where sulfur is oxidized (26). The microörganisms in the soil are also able to convert easily soluble phosphates into difficultly soluble and vice versa (39).

EXPERIMENTAL METHODS

Soil treatments

The soil treatments were carried out in porcelain dishes in the laboratory. It was found desirable that the system soil:water should be as close to natural conditions as uniform and adequate conditions for interaction between the soil and the applied phosphate would permit. For this reason, saturation of the phosphate treated soil samples with distilled water was adopted. Further experimental details as to treatment of the soils are given under each series.

Analytical methods

pH values were determined by means of the quinhydrone electrode, using 1:1.5 soil:water ratio.

Readily soluble phosphate. The $KHSO_4$-method of extraction described in a previous paper (28) was used in the determination of readily soluble phosphate in the soil samples. Two grams of air-dry 20-mesh soil is shaken for 5 minutes with 400 cc. of dilute potassium acid sulfate solution at a pH of 2.00. The extract is filtered at once and phosphorus determined colorimetrically. This method has several important advantages for the study of readily soluble phosphate in soils. Because of the short time of extraction, only the more readily soluble phosphates are dissolved out of the soil; the difficultly soluble phosphates are not attacked to any appreciable extent. The H-ion concentration of the solvent (pH 2.00) is such that precipitation of dissolved phosphate does not take place and the buffer capacity of the solution is such that the pH of the extract remains practically unchanged (pH 2.00, pH 2.05).

Since all the soils are extracted at practically constant pH, the results of the determinations are on a uniform basis for comparison.

The authors' contention that this method extracts practically only the readily soluble phosphate from a soil is supported by the following results from the extraction of several known phosphates.

One gram of each phosphate was shaken with 400 cc. of $KHSO_4$ solution, pH 2.00, for 5 minutes and the extract filtered at once through a prepared Whatman #42 filter paper in a 9½-cm. Buchner funnel. Phosphorus in the filtrate was determined colorimetrically by the Fiske and Subbarow Method (14) and the readings were made on a Klett Bio-Colorimeter.

PHOSPHATE	PER CENT OF THE TOTAL PHOSPHORUS CONTENT EXTRACTED BY THE $KHSO_4$ METHOD	pH IN EXTRACT*
Mono-calcium phosphate, $CaH_4(PO_4)_2 \cdot H_2O$ (P. W. R.)	100	2.19
Di-calcium phosphate, $CaHPO_4 \cdot 2H_2O$ (Baker C. P.)	93	3.18
Tri-calcium phosphate, $Ca_3(PO_4)_2$ (Merck, precipitated)	44	2.26
Aluminum phosphate, $AlPO_4$ (Merck, C.P.)	7	2.43
Ferric phosphate, $FePO_4 \cdot 4H_2O$ (Merch)	3	1.99
Canadian apatites (green apatite; sugar apatite) (100-mesh)	3	2.16; 2.26

* The pH-values were measured by means of a quinhydrone-electrode, and a saturated calomel cell. The measurements were carried out in a period, where the temperature changed very much.

From the foregoing results it would seem that the $KHSO_4$ method is well adapted for use in determining the readily soluble phosphate content of the treated soils in the following series of experiments. It is very unlikely that this method results in the extraction to an appreciable extent of the more difficultly soluble phosphates resulting from conversion or fixation of soluble phosphates added to the soil.

SERIES I

Procedure. The soil used was an acid clay from the Welland Fertility Experimental Field check plot. Air-dry, 20-mesh soil, in portions of 202 gm. each, was placed in 500 cc. porcelain dishes, the various amounts of superphosphate and rock phosphate were added, the soils and fertilizers were mixed, and 125 cc. distilled water was added to each dish (saturation). The soils were allowed to dry at room temperature. When dry, the soil in each dish was gently ground up, mixed, and sampled for analysis. The saturation with water, drying, and sampling were repeated twice.

Data. The results of the determinations of readily soluble phosphate in each of the samples after each wetting and drying are given in table 1.

It is remarkable that even the amount of soluble phosphate supplied by the superphosphate at 1,200 pounds per acre has not increased the readily soluble phosphate content of this soil at the end of the experiment. Rock phosphate, however, at the rates of 1,000 and 2,000 pounds per acre does increase the readily soluble phosphate content of the samples, as would be expected, since the total amount of phosphorus supplied is much higher than in the case of the superphosphate treated samples. It is interesting to note that 500 pounds of rock phosphate per acre has about the same effect on the readily soluble phosphate content as 1,200 pounds of superphosphate. In the case of the subsoil samples, 1,000 pounds of rock phosphate per acre is more effective in increasing the readily soluble phosphate content than is superphosphate at 1,200 pounds per acre.

TABLE 1

Effect of superphosphate and rock phosphate on readily soluble phosphate content of Welland clay—series I

	P EXTRACTED PER 1,000 GM. OF SOIL ($KHSO_4$-METHOD)		
	After 1 moistening (1 week)	After 2 moistenings (3 weeks)	After 3 moistenings (6 weeks)
	mgm.	*mgm.*	*mgm.*
Surface soil (pH 5.22):			
1. No phosphate	39	...	...
2. 200 pounds 16 per cent superphosphate* per acre	38	34	32
3. 400 pounds 16 per cent superphosphate per acre	38	36	29
4. 1,200 pounds 16 per cent superphosphate per acre	50	41	34
5. 500 pounds 31 per cent rock phosphate per acre	52	49	37
6. 1,000 pounds 31 per cent rock phosphate per acre	61	53	50
7. 2,000 pounds 31 per cent rock phosphate per acre	114	109	83
Subsoil (pH 5.63):			
8. No phosphate	13	...	...
9. 1,200 pounds 16 per cent superphosphate per acre	30	24	11
10. 1,000 pounds 31 per cent rock phosphate per acre	55	45	25

* The acre-furrow slice calculated as 2,000,000 pounds of soil.

Similar experiments with a calcareous phosphate-deficient soil (not reported here) gave the same results as the foregoing. The superphosphate used in this series was in a fine state of subdivision. The authors have experimented with several "granulated" superphosphates and it has been found that granulation of the superphosphate retards its conversion in the soil to a considerable extent.[4]

SERIES II

Two soils were used in this series: an acid clay soil from Kent County and a medium sand from Norfolk County, Ontario. Several experiments were carried out in order to find a suitable standard technic for making the soil treatments. The following procedure was finally adopted:

Phosphate at the rate of 300 mgm. P per 1,000 gm. of soil[5] was mixed with 300 gm. air-dried soil (20 mesh) and placed in a 500 cc. porcelain dish. The soil was then saturated with distilled water and stirred thoroughly with a glass rod in order to ensure intimate mixing of the phosphate with the soil. The samples were then placed in a room at 20–22°C. and allowed to dry out, which took about a week. The air-dried samples were ground very gently and mixed thoroughly before extraction by the $KHSO_4$-method. In order to follow the conversion of the phosphates, the soil samples were saturated with water again, and after 5 days, water was added as follows: 50 cc. to the clay and 30 cc. to the sand. The series were left to dry out at room temperature and extracted (see columns II and III, tables 2 and 3). With a few exceptions, all of the treatments were carried out in duplicate. The acidity of all samples was determined after the third moistening (ratio soil: water = 1:1.5).

Data. The results of these experiments are given in tables 2 and 3. A study of the data in tables 2 and 3 shows that in the clay soil, soluble phosphates are converted into difficultly soluble phosphates to a much greater extent than in the sand.

It is a remarkable fact that the secondary phosphates (except for the sodium phosphates) show least conversion. Attention is also called to the changes in reaction of the phosphate-treated samples, as compared with the untreated control.

SERIES III

Procedure. In this experiment a study was made of the influence of different basic materials on the solubility of mono-calcium-phosphate added to the clay soil from Kent County which had been used in the previous experiment. Increasing amounts of several basic materials were added to 300-gm. portions of this soil. Calculations on the acre basis were made

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[4] In comparing these results with superphosphate and rock phosphate, it must be borne in mind that the total amounts of phosphorus added to the soil samples are about twice as high for the samples treated with rock phosphate as for the samples treated with superphosphate.

[5] If the surface horizon of one acre of land is arbitrarily estimated to weigh 1,000,000 kgm., 300 mgm. P per 1,000 gm. of soil will correspond to an application of 300 kgm. P per acre. This amount is, of course, much larger than any amount of phosphate used in fertilizer practice. It is, however, common practice to drill the fertilizers in close proximity to the seed, and further granulated fertilizers are widely used. Thus, the amount of phosphate fertilizer applied to an acre of land cannot be considered to react intimately with the whole amount of soil in the plowed layer. *The reaction of the fertilizer salts with the soil must be definitely localized, and it must be considered to be a reaction of a highly concentrated solution with the soil compounds.* To the knowledge of the authors, this theory was first advanced by Fraps (15).

TABLE 2

Phosphate experiment with different synthetic phosphates added to a surface soil from Charing Cross, Kent County—series II*

PHOSPHATE ADDED	P ADDED PER 1,000 GM. OF SOIL	P EXTRACTED PER 1,000 GM. OF SOIL (KHSO₄-METHOD)			pH IN SOIL AFTER LAST MOISTENING
		I	II	III†	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
No phosphate	0	18	19	13	6.20
$CaH_4(PO_4)_2 \cdot H_2O$	300	96	100	84	6.13
$CaHPO_4 \cdot 2H_2O$	300	171	146	112	6.50
$Ca_3(PO_4)_2$	300	49	58	57	6.19
$MgHPO_4 \cdot 3H_2O$	300	106	82	70	6.53
$Mg_3(PO_4)_2 \cdot 4H_2O$	300	71	63	46	6.65
$BaH_4(PO_4)_2$	300	100	84	65	6.23
$BaHPO_4$	300	123	88	71	6.50
$Mn_3(PO_4)_2 \cdot 2H_2O$	300	108	83	63	5.91
$AlPO_4$	300	55	62	62	6.21
$FePO_4 \cdot 4H_2O$	300	37	55	63	6.17
$(NH_4)H_2PO_4$	300	98	76	78	6.33
$(NH_4)_2HPO_4$	300	107	87	82	6.42
KH_2PO_4	300	78	94	73	6.13
K_2HPO_4	300	103	101	82	6.32
K_3PO_4	300	81	88	62	6.53
$NaH_2PO_4 \cdot H_2O$	300	168	146	131	6.59
$Na_2HPO_4 \cdot 12H_2O$	300	138	104	87	6.94
$Na_3PO_4 \cdot 12H_2O$	300	123	124	110	7.37
$NaNH_4 \cdot HPO_4 \cdot 4H_2O$	300	99	98	84	6.60

* Light gray-brown clay loam.

† I—150 cc. H_2O; II—150 + (149 + 50) 349 cc. H_2O; III—349 + (148 + 50) = 547 cc. H_2O.

TABLE 3

Phosphate experiment with different synthetic phosphates added to a surface soil from the tobacco plots in Norfolk County—series II*

PHOSPHATE ADDED	P ADDED PER 1,000 GM. OF SOIL	P EXTRACTED PER 1,000 GM. OF SOIL (KHSO₄-METHOD)			pH IN SOIL AFTER LAST MOISTENING
		I	II	III†	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
No phosphate	0	23	19	21	6.26
$CaH_4(PO_4)_2 \cdot H_2O$	300	165	170	168	6.01
$CaHPO_4 \cdot 2H_2O$	300	285	285	297	6.15
$MgHPO_4 \cdot 3H_2O$	300	172	178	180	6.29
$(NH_4)_2HPO_4$	300	162	168	168	6.45
KH_2PO_4	300	162	162	167	5.94
K_2HPO_4	300	174	174	182	6.51

* Fox coarse sand.

† I—90 + 30 = 120 cc. H_2O (dried out very rapidly, therefore moistened again after 5 days); II—120 + (89 + 30) = 239 cc. H_2O; III—239 + (88 + 30) = 357 cc. H_2O.

by assuming that the surface layer on 1 acre land weighs 1,000,000 kgm. The basic materials were mixed with the air-dry soil and 150 cc. H_2O added (saturation), whereafter the wet soil was thoroughly stirred with a glass rod. Further 50 + 50 cc. H_2O were added at intervals of 4 days, and the soils were left to dry out at room temperature. The soil was then gently ground up, and monocalcium-phosphate was added at a rate of 300 mg. P per 1000 gm. of soil, 150 cc. H_2O was added again and the wet soil stirred thoroughly. At intervals of 4 days, further 50 + 50 + 50 cc. H_2O were added and the soils air-dried. The large amounts of water were used in this experiment in order to insure an efficient interaction between the basic materials and the phosphate.

Although there is a reduction in the acidity of the soils receiving basic material with the phosphate, the amounts of readily soluble phosphate extracted have not been greatly increased over those obtained where the phosphate was used alone (table 4). Further, the different basic materials show little difference in their individual effect on the soluble phosphate content.

TABLE 4

Phosphate experiment with $CaH_4(PO_4)_2 \cdot H_2O$ with a surface soil (Charing Cross, Kent County) supplied with increasing amounts of basic materials—series III

BASIC MATERIAL, TONS PER ACRE	P ADDED PER 1,000 GM. OF SOIL	P EXTRACTED PER 1,000 GM. OF SOIL ($KHSO_4$-METHOD)	pH IN SOIL AFTER LAST WETTING
	mgm.	*mgm.*	
$CaH_4(PO_4)_2 \cdot H_2O$	300	107	5.95
2 tons dolomitic limestone	300	116	6.95
5 tons dolomitic limestone	300	123	7.10
10 tons dolomitic limestone	300	130	7.23
2 tons $CaCO_3$ (powdered)	300	126	7.04
5 tons $CaCO_3$ (powdered)	300	129	7.44
10 tons $CaCO_3$ (powdered)	300	132	7.68
2 tons calcium silicate (synthetic)	300	98	6.50
5 tons calcium silicate (synthetic)	300	117	7.05
10 tons calcium silicate (synthetic)	300	122	7.47

Similar experiments have been conducted by Hall and Vogel (20). Parker and Tidmore (32) found an increase in the concentration of phosphorus in the soil solution from superphosphate treated soils, which had received, also, increasing amounts of lime.

SERIES IV

Procedure. The influence of different fertilizer salts on the solubility of mono- and dicalcium phosphate added to the clay soil, Kent County, was studied as follows: 250 gm. air-dry soil was weighed out in 500 cc. porcelain dishes and by means of the edge of a 250 cc. beaker a circular furrow was made in the center of the soil in the dish. The phosphates were added at the rate of 300 mgm. P per 1,000 gm. of soil, and distributed evenly in the furrow; then the fertilizer salts to be added were weighed out and were distributed evenly in the same furrow. The remaining 50 gm. of soil was then added as a cover for the fertilizer salts, a total of 300 gm. soil thus being used. In this way, an attempt was made to approximate more nearly the conditions for row-application and granulation of fertilizers. Each dish of soil received 150 cc. H_2O (saturation), but it was not stirred wet, as was the case in the former experiments. After the elapse of 5 days, a further 50 cc. H_2O was added, and the samples were left to air-dry. The air-dry samples were gently ground up and mixed thoroughly by energetic stirring of the pulverized soil with a rubber pestle in an iron mortar. The duplicates gave good checks, which proves that the mixing had been effective. The synthetic salts of $NaNO_3$, $(NH_4)_2SO_4$, $Ca(NO_3)_2 \cdot 4H_2O$, $MgSO_4$, KCl and K_2SO_4 were added in such amounts that the bases in these salts were equivalent to the calcium in the dicalcium phosphate. The results are reported in table 5.

TABLE 5

Effect of salts on the solubility of mono- and di-calcium phosphates added to the surface soil from Charing Cross, Kent County—series IV

PHOSPHATE	SALT ADDED	pH PER 1,000 GM. OF SOIL ($KHSO_4$-METHOD)	pH IN SOIL AFTER TREATMENT (1:1.5 SUSPENSION)
		mgm.	
No phosphate	None	18	6.13
$CaH_4(PO_4)_2 \cdot H_2O$	None	132	6.06
$CaH_4(PO_4)_2 \cdot H_2O$	$NaNO_3$	132	5.85
$CaH_4(PO_4)_2 \cdot H_2O$	$(NH_4)_2SO_4$	116	5.73
$CaH_4(PO_4)_2 \cdot H_2O$	KCl	122	5.52
$CaH_4(PO_4)_2 \cdot H_2O$	K_2SO_4	129	5.63
$CaH_4(PO_4)_2 \cdot H_2O$	$Ca(NO_3)_2 \cdot 4H_2O$	141	5 50
$CaH_4(PO_4)_2 \cdot H_2O$	$MgSO_4$	129	5.70
$CaH_4(PO_4)_2 \cdot H_2O$	$CO(NH_2)$ (966 mgm. per 1000 gm. soil)	153	6.75
$CaH_4(PO_4)_2 \cdot H_2O$	Calcium cyanamid, granulated (980 mgm. per 1,000 gm. soil)	206	6.76
$CaH_4(PO_4)_2 \cdot H_2O$	Calcium cyanamid, granulated (1,960 gm. per 1,000 gm. soil)	213	7.21
$CaH_4(PO_4)_2 \cdot H_2O$	Calcium cyanamid, non-granulated (980 mgm. per 1,000 gm. soil)	186	6.76
$CaH_4(PO_4)_2 \cdot H_2O$	Dolomitic limestone (1,667 mgm. per 1,000 gm. soil)	159	6.16
$CaH_4(PO_4)_2 \cdot H_2O$	Dolomitic limestone (3,333 mgm. per 1,000 gm. soil)	180	6.24
$CaHPO_4 \cdot 2H_2O$	None	324	6.18
$CaHPO_4 \cdot 2H_2O$	$NaNO_3$	306	6.19
$CaHPO_4 \cdot 2H_2O$	$(NH_4)_2SO_4$	282	5.70
$CaHPO_4 \cdot 2H_2O$	KCl	303	5.77
$CaHPO_4 \cdot 2H_2O$	K_2SO_4	306	5.83
$CaHPO_4 \cdot 2H_2O$	$Ca(NO_3)_2 \cdot 4H_2O$	309	5.82
$CaHPO_4 \cdot 2H_2O$	$MgSO_4$	312	5.98
$CaHPO_4 \cdot 2H_2O$	$CO(NH_2)$ (966 mgm. per 1,000 gm. soil)	321	6.95
$CaHPO_4 \cdot 2H_2O$	Calcium cyanamid (980 mgm. per 1,000 gm. soil)	309	6.76
$CaHPO_4 \cdot 2H_2O$	Dolomitic limestone (1,667 mgm. per 1,000 gm. soil)	303	6.31

A study of the data in table 5 further emphasizes the difference in recovery of soluble phosphate when mono- and di-calcium phosphates are used alone and in combination with other salts. In all cases, the amounts of readily soluble phosphate extracted from the dicalcium phosphate treated samples are greater than the amounts extracted from mono-calcium phosphate treated samples. The di-calcium phosphate apparently has been much less converted than has the mono-calcium phosphate.

SERIES V

Procedure. Different fractions of granulated 16 per cent superphosphate[6] were carefully mixed with 300 gm. air-dry soil (clay soil from Kent County).

The water supply in this series was as follows:

SUB-SERIES	ADDED H_2O	NUMBER OF DAYS MOIST
	cc.	
I	150 (saturation) + 50 = 200	6
II	200 + 4 × 50 = 400	15
III	400 + 4 × 50 = 600	24

The phosphate was added in a rate of 300 mgm. readily soluble phosphate per 1,000 gm. of soil[7] this being determined by extracting the superphosphate with an acid KCl solution (0.1 *N* KCl containing 1.4104 gm. HCl per liter; pH-1.48; ratio of solid to solvent 1:500). The procedure for grinding and mixing of the dry soil was as in series IV.

TABLE 6

Experiment with granulated superphosphate (soil from Charing Cross, Kent County)—series V

FRACTION	P ADDED PER 1,000 GM. SOIL (SOLUBLE IN 0.1 *N* KCl + HCl SOLVENT) (pH 1.48)	P EXTRACTED PER 1,000 GM. SOIL ($KHSO_4$-METHOD)		
		I	II	III
	mgm.	*mgm.*	*mgm.*	*mgm.*
1. Larger than 10 mesh	300	174	135	126
2. 10 mesh	300	162	129	126
3. 20 mesh	300	153	126	125
4. 40 mesh	300	147	126	126
5. 60 mesh	300	129	126	125
6. No phosphate	0	...	...	14

It is seen from table 6 that in the 60-mesh mixture, the soluble phosphate is at once converted to its full extent under the experimental conditions here, whereas the conversion of the phosphate from the larger granules has been retarded the more, the bigger the granules are. In this connection it is interesting to note that the granules from the larger fractions were still intact at the time the samples were analyzed. Apparently the mono-calcium phosphate becomes leached out of the granules and the conversion thus is dependent on the rate at which this leaching takes place.

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[6] The superphosphate used in this experiment is widely used in Canada. Experiments with another granulated phosphate, also widely used in Canada, yielded similar results.

[7] The total amount of P added was 326 mgm. P per 1,000 gm. of soil.

INTERPRETATION OF DATA

In considering the data presented, it must be emphasized that they should be interpreted only on a *solubility* basis. In the opinion of the authors, the true value to the plant of the undissolved phosphate remaining in the soil after extraction of the samples can be estimated, at the present time, only by permanent field experiments carried on for such a time that the residual effect of the phosphate treatment is measured.

It is seen from the experimental data that *all* soluble phosphates are more or less converted into more difficultly soluble phosphates in the soils. Also with rock phosphate, the solubility is decreased by prolonged moistening. This might be because the finest particles dissolve most readily and the dissolved phosphate becomes converted in the soil. Also, it might be possible that the phosphate particles become more or less coated with hydrogels of iron and alumina.

The di-calcium phosphate has not been converted very much because this salt is slowly decomposed by water and it is relatively stable under the acidity conditions prevailing in the soil samples studied.

The comparatively low conversion of the sodium phosphates may be ascribed to the fact that sodium reacts readily with the humus in the soil to form soluble humates (13). Midgley (31) also found that sodium phosphate was only slightly converted in the soil. As far as the water-soluble phosphates are concerned, their conversion is readily understood when we recall the fact that an excess of soil bases (Fe, Al, Ca, Mg) (16) in the acidity range of pH about 3 to about 6.5 will cause almost complete precipitation of phosphate from a solution. Such conditions will exist in most soils, as even large amounts of soluble phosphate applied as in the experiments here will mean that the bases in the soil are in large excess of the soluble phosphate added. Also in considering titration curves of the phosphates important in soil studies (4), it becomes very obvious that acid phosphates (mono-phosphates) are readily converted. Superphosphates, for instance, possess a very high acidity, and as soon as they are dissolved in the soil moisture, they are bound to be converted into phosphates stable under the acidity conditions of the soil. As far as the synthetic difficultly soluble phosphates, such as $Ca_3(PO_4)_2$, $AlPO_4$, and $Fe(PO_4) \cdot 4H_2O$, are concerned (see table 2), it is noteworthy that they have increased in solubility as the time of moistening has become prolonged.

Organic matter seems to have a marked effect in preventing or retarding conversion of soluble phosphates into more difficultly soluble compounds. In fact, in some tentative experiments where very small amounts of organic substances, such as *dextrose*, *glycerine*, *gelatine*, and *phenylhydrazine* were added, the decrease in solubility of mono-calcium phosphate added to the soil from Kent County, was markedly less than where no organic substances were added.

As far as mono-calcium phosphate is concerned, chemical studies on the calcium phosphates have conclusively proved that its existence in any soils as mono-phosphates is excluded (6, 2, 4, 16, 21). The di-calcium phosphate, which is sparingly soluble in water, does not seem to be as readily converted into more difficultly soluble soil phosphate. This is of some significance, as it is considered to be fully available for the plants. Its conversion into more basic calcium phosphates has been studied by Buch (5). The conversion into tri-calcium phosphate is, according to Buch, possible after the following scheme:

$$CaHPO_4 \rightleftharpoons Ca^{++} + HPO_4{}^{=} \tag{A}$$

$$3HPO_4 \rightleftharpoons 2PO_4{}^{\equiv} + H_3PO_4 \tag{B}$$

By further hydrolysis the tri-calcium phosphate becomes converted into hydroxy-apatite (Warrington, 40, 41):

$$10\ Ca_3(PO_4)_2 + 6H_2O = 3\ [3Ca_3(PO_4)_2 \cdot Ca(OH)_2] + 2H_3PO_4$$

Holt, la Mer, and Chown (21) have shown by lime titrations of phosphoric acid that the formation of the di-calcium phosphate takes place readily, as it is a second order reaction, whereas the conversion of di-calcium phosphate into tri-calcium phosphate is slow, because the formation of the tri-calcium phosphate is a fifth order reaction, and the solution can remain super-saturated with respect to tri-calcium phosphate for many days. These conditions are illustrated by the following scheme:

$$Ca^{++} + HPO_4{}^{=} \rightleftharpoons CaHPO_4 \tag{A}$$

$$3\ Ca^{++} + 2PO_4{}^{\equiv} \rightleftharpoons Ca_3(PO_4)_2 \tag{B}$$

Another factor which may, under certain circumstances, serve as a retarder for the di-calcium phosphate is the fact that particles of di-calcium phosphate can be covered with a gelatinous coating of tri-calcium phosphate (1). This phenomenon has also been observed by one of the authors (H. W. L.) in studies on the retardation of the disintegration rate of granulated phosphatic mixtures. Britton (4) showed, on the basis of Bassett's data, that di-calcium phosphate can exist as such in aqueous solutions of a pH range of 3 to 5.5. Thus, the tension of CO_2 in the soil water under the circumstances, may determine the existance of the di-calcium phosphate, which may be illustrated as follows:

$$2\ CaHPO_4 + Ca^{++} + 2\ HCO_3{}^{-} \rightleftharpoons 2\ H_2O + 2\ CO_2 + Ca_3(PO_4)_2$$

Some explanation regarding the conversion of the water-soluble phosphates in soils may be gained from the fact that they can be precipitated by calcium salts (23). The primary—but not the secondary—phosphates will be precipitated by calcium salts of weak acids:—

$$2\ NaH_2PO_4 + 3\ CaCO_3 \rightleftharpoons Ca_3(PO_4)_2 + Na_2CO_3 + 2H_2O + 2CO_2$$

The secondary—but not the primary—phosphates will be partially precipitated by calcium salts of strong acids:—

$$4\ Na_2HPO_4 + 3\ CaSO_4 \rightleftharpoons Ca_3(PO_4)_2 + 2NaH_2PO_4 + 3\ Na_2SO_4$$

Kugelmass and Shohl (24) have studied the quantitative equilibrium relations for the ions: $Ca^{++}, H^{+}, HCO_3, ^{-}CO_3{}^{=} HPO_4{}^{=}, H_2PO_4{}^{-}$, and $PO_4{}^{\equiv}$ at 38°C., a study which also is important for the understanding of the problems discussed here.

In the discussion of the experimental data presented, biological processes in the soil samples have not been taken into account. It might be possible, of course, that the activity of microörganisms in these soils has influenced the results to some extent.

The general conclusion which can be drawn on the basis of the data reported is that the ability of a given phosphate to increase the amount of phosphate extractable by the method employed depends partly upon the solubility and nature of the phosphate, and partly upon the power of the soil to convert the dissolved phosphate into a more difficultly soluble form. This suggests that the efficiency[8] of water-soluble phosphates in increasing the phosphate supply of soils for the plants depends upon how far their conversion is retarded or, what should be better, is prevented [see also (12)].

The fact that the di-calcium phosphate is practically fully extracted when conversion is prevented (see Series 4) suggests that the extraction method used is a suitable one, as this phosphate is available for the crops. Also, it becomes apparent that phosphates of this type should possess an efficiency of about 100 per cent when applied in a suitable way.[9]

From the data reported, it becomes apparent that studies on the conversion of phosphates in soils should accompany extraction studies when this problem is studied for agricultural purposes. Also, where field and laboratory experiments in phosphate fertility are to be compared, such conversion studies should always be carried out, and this especially where phosphate fertilizers are distributed a long time before the crops are sown, or in studies with crops which absorb the bulk of their nutrients late in the growing season.

SUMMARY

A technic for the determination of the ability of a soil to convert soluble phosphates, added to it has been developed.

Extraction studies have been made on phosphate-treated soil samples.

The effect of basic materials, fertilizer salts, and the method of incorporating the phosphate in soils upon the solubility of some phosphates added to soils has been studied.

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[8] This term is adopted from Conner (10).

[9] Fertilizer experiments with mono- and di-calcium phosphates have been carried out by Scharrer and Strobel (36).

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THE LAWS OF SOIL COLLOIDAL BEHAVIOR: XIII. OSMOTIC IMBIBITION[1]

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The water which is adsorbed by soil colloids in addition to their hygroscopic water and which under certain conditions (as when the colloid is saturated with Na) causes the material to swell greatly, has been defined as the water of osmotic imbibition (3). This water differs from the hygroscopic moisture in that it does not manifest any measurable heat of wetting. Its vapor tension is not lower than that of common salt solutions, and it is therefore evaporated in ordinary air. This is also brought out by the fact established by Lebedeff (2) that the relative humidity in the soil air is 100 when the soil is hygroscopically saturated. The forces by which this water is held are therefore nor nearly so great as the molecular forces of attraction which bind the hygroscopic moisture.

The following qualitative and quantitative relationships of the water of ismotic imbibition have been established and were discussed in some of the previous publications (3, VIII):

1. The amount of water imbibed and the swelling produced are (for a given cation) pro portional to the cation exchange capacity of the colloid and depend therefore upon the composition of the latter. If the proportion of silica is high and the cation exchange capacity at pH 7.0 is therefore also high the osmotic imbibition will be greater than in the case of colloids having a high sesquioxide content and possessing a low cation exchange capacity (3, V).
2. Since the imbibition of water and the resulting swelling are related to the composition of the colloid and its cation exchange capacity at pH 7 0 they are also related to the amphoteric nature and the electrokinetic behavior of the colloid: its isoelectric point, its pH of exchange neutrality, and its ultimate pH (3, VI).
3. The imbibition and swelling vary greatly with the nature of the exchangeable cation, being greatest with Na, intermediate with K, and small with H and Ca (3, VIII).
4. Since the imbibition varies with the nature of the diffusible and exchangeable cations and since the charge of the particles likewise varies in the same order with the nature of these cations, we might say that the imbibition and swelling are also related to the charge.
5. If the unsaturated colloid (electrodialyzed, H-saturated) be saturated with a cation which produces a greater swelling (Na) the swelling or imbibition will reach a maximum and this maximum coincides with the exchange capacity of the colloid. With an excess of base (NaOH) the swelling is suppressed and the more so the greater the excess (3, VIII).
6. Any other electrolyte which is added to the colloid will suppress the swelling. This

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[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

suppression of the swelling caused by a free electrolyte is governed by the concentration and by the valence of either of the ions as demanded by the Donnan equilibrium. This suppressing effect must not be confused with the suppression resulting when an ion producing a great swelling is displaced by an ion causing a moderate or slight swelling. The effect might then be much greater, but the phenomenon has nothing to do with the Donnan equilibrium (3, I).

7. The pressure required to remove the imbibed water was found to be inversely proportional to the cube of the water content. From the work of Bouyoucos (1) it appears that the depression of the freezing point bears the same relationship to the water content (3).

8. The imbibed water does not contain as many of the free diffusible ions not in combination with the colloid (anions in the case of a negative colloid) as the outside solution in equilibrium with the gel. There is a "negative adsorption" of the ions of the free electrolyte. This unequal distribution of the ions is governed by the concentration and by the valence of the ions as demanded by the Donnan equilibrium, which for a univalent salt is expressed by the equation

$$x^2 = y\,(y + z)$$

where x is the concentration of each of the ions of the free electrolyte in the outside (not imbibed) solution and y the concentration of these ions within the imbibed solution whereas z is the concentration of the diffusible ions (cations in the case of a negative colloid) in the imbibed water which are dissociated by the colloidal particle or ionic complex and around which they form a swarm or ion atmosphere held by the electrostatic attraction (3, I).

9. The lower the moisture content of the gel below that of its maximum imbibition the greater is the negative adsorption or, in other words, the smaller is the concentration y as compared to x (3, I).

10. The solution removed from the gel at high pressures has a lower concentration than the solution removed at low pressures (3, VIII).

These observations have led to the following conception of the mechanism of imbibition and swelling of soil colloids:

The colloidal particle dissociates some of the diffusible ions in combination. The degree of dissociation depends upon the nature, valence, and hydrations of the ions. Hence the lyotropic series. The dissociated ions form an atmosphere around the particle which latter constitutes a highly multivalent colloidal ion of opposite charge to that of the diffusible ions. The concentration z of the dissociated ions is greatest near the surface of the particle resulting in a steep concentration gradient perpendicular to the surface. The thickness of the ion atmosphere is limited by the electrostatic attraction. Within the ion atmosphere the osmotic pressure is greater than in the outside liquid and grows progressively greater in the direction of the surface. (The pressure increases apparently in an inverse ratio to the sixth power of the distance from the surface.) The greater the osmotic pressure the greater will be the force which is required to remove the imbibed water. The colloidal particle, the ion atmosphere, and the osmotically imbibed water constitute the colloidal micelle.

The imbibition of water is therefore a function of an ionic condition of the colloid, in other words, of a colloidal ionogen. The Na-saturated colloid swells more than the K- and Ca-saturated because the Na ions are more dissociated by the complex and thus give rise to a higher osmotic pressure in the micellar solution. The smaller Na ions possess originally a greater self potential than the larger univalent cations such as the K ions. The former attract therefore the bipolar water molecules more strongly and become more heavily hydrated than the latter. This hydration leads to a partial suppression of the activity or self potential of the ions, and the Na ions have therefore in reality a lower potential and are less attracted by the opposite charge on the colloidal particle or ionic complex. The Na ions among the common ions in the soil are therefore not only most highly dissociated by the complex but are in addition able to diffuse farther out into the dispersion medium, since a higher potential difference in the double layer is required to hold these ions in check. The ion atmosphere is therefore thicker and the osmotic imbibition greater in a Na-saturated colloid. For the same reason the electrokinetic potential of the particles, which is proportional to the number of free charges resulting from dissociation and the distance which the dissociated ions diffuse into the surrounding medium, is greatest in the case of the Na-saturated colloid. Thus the Na ions, which by themselves migrate more slowly in the electric field than the other common ions, impart the greatest cataphoretic velocity to the colloidal particles.

The divalent cations, some of which are highly hydrated, form more strongly associated compounds with the soil colloids The critical potential, which limits the dissociation and the outward diffusion of the dissociated ions, is lower for the divalent ions. The osmotic imbibition, swelling, and charge of colloids saturated with these ions are therefore also lower. This must evidently be ascribed to their double charge.

EXPERIMENTAL

In the previous experiments on the pressure required to remove water from bentonite at various water contents of the gel the material was employed in its naturally occurring condition. It was decided to extend this work to include bentonite saturated with the different common cations as has been done in the experiments on swelling, dispersibility, cataphoresis, etc. (3, I, II, VIII).

Another sample of bentonite from Rock Creek, Wyoming, was obtained. This time the material was freed from sand and silt by sedimentation. The bentonite was then electrodialyzed in a large cell of the Mattson type made from pine wood and impregnated with paraffin and having a capacity of about 2 kgm. soil.

The influence of the nature of the exchangeable cation

To a series of suspensions of the electrodialyzed bentonite equivalent quantities of lithium and potassium hydroxides and of magnesium and calcium oxides were added. The total quantity added was determined by the exchange capacity of the colloid. The original bentonite represents primarily a sodium-saturated product. The samples saturated with the different cations and a portion of the electrodialyzed (H-saturated) material were air dried, passed through a 100-mesh sieve, and then redispersed in sufficient water to make a thick yet fluid suspension. The relationship between pressure and water content was then studied.

To do this, samples of the gels were subjected to various vacuum pressures until equilibrium was reached. The different pressures were arrived at, by fitting to suction flasks, glass funnels with fused-in sintered porcelain filters. To evacuate the suction flasks, they were attached to an electrically driven vacuum pump. The vacuum pressures exerted on these gels were measured and adjusted by means of a mercury manometer. The pressure on the gel in any one specific funnel could in this way be regulated and kept uniform until equilibrium was reached; that is, until the force exerted by the bentonite for the moisture was equal to the vacuum pressure tending to withdraw the moisture. These opposing forces were assumed to be equal when no perceptible solution was withdrawn from the gel within a period of 24 hours. It usually required about 10 days to reach this condition of equilibrium stability.

To prevent drying out of the gel by evaporation, the humidity of the air surrounding the gel was kept uniform by placing the suction flasks together with the funnels and their gel contents within a closed glass container over an open water bath.

TABLE 111

Volume of water retained per gram of natural bentonite against various vacuum pressures

PRESSURE	WATER RETAINED PER GRAM BASIS 110°C.	(VOLUME) (PRESSURE$^{1/3}$)
cm. Hg	*cc.*	
70	3.248	13.384
60	3.462	13.555
30	4.307	13.384
30	4 445	13.813
20	4.795	13.015
15	5.649	13.933
5	7.914	13.533
5	8.0101	13.697
Gravity	9.508	

Arithmetic mean.. 13.539 ±0.181
Standard deviation.. 0.269
Equation connecting variables:

$$\text{Log } P = -3 \text{ Log } V + 3 \text{ Log } 13.539 \pm 0.181$$

$$dp/dv = -3$$

TABLE 112

Volume of water retained per gram of electrodialyzed bentonite (not air-dried since first dispersed) against various vacuum pressures

PRESSURE	WATER RETAINED PER GRAM BASIS 110°C.	(VOLUME) (PRESSURE$^{1/3}$)
cm. Hg	*cc.*	
70	1.786	7.349
60	1.892	7.407
50	2.121	7.812
30	2.412	7.493
20	2.833	7.690
Gravity	6.98	

The results of the experiments are shown in tables 111 to 117.

In the original experiments on the relationship between the pressure and the water content of the gel in which untreated bentonite (from which no sand was separated by sedimentation) was used, the products obtained by multiplying the volume of the water retained by the cubic root of the applied pressure deviated but slightly from 10.70 within the pressure range from 5.6 to 70

TABLE 113

Volume of water retained per gram of electrodialyzed bentonite (air-dried after electrodialyzing) against various vacuum pressures

PRESSURE	WATER RETAINED PER GRAM BASIS 110°C.	(VOLUME) (PRESSURE$^{1/3}$)
cm. Hg	*cc.*	
60	0.762	2.987
60	0.754	2.958
60	0.782	3.065
50	0.802	2.954
50	0.788	2.902
40	0.828	2.828
40	0.852	2.914
38	0.862	2.898
30	0.976	3.030
30	0.914	2.841
20	1.033	2.805
20	1.040	2.824
14	1.252	3.013
12	1.250	2.861
10	1.465	3.157
10	1.527	3.289
8	1.431	2.861
7	1.790	3.43
4	2.056	3.263
3	2.192	3.162

Arithmetic mean.. 3.021 ±0.116

Standard deviation.. 0.172

Equation connecting variables:

$\text{Log } P = -3 \text{ Log } V + 3 \text{ Log } 3.021 \pm 0.116$

$dp/dv = -3$

TABLE 114

Volume of water retained per gram of air-dried Mg-saturated bentonite against various vacuum pressures

PRESSURE	WATER RETAINED PER GRAM BASIS 110°C.	(VOLUME) (PRESSURE$^{1/3}$)
cm. Hg	*cc.*	
50	0.816	3.003
50	0.850	3.130
40	0.907	3.10
40	0.868	2.97
30	0.963	2.992
30	0.950	2.953
25	1.044	3.053
25	1.033	3.021
20	1.076	2.922
20	1.091	2.962
Gravity	2.456	

Arithmetic mean.......................... 3.011 ±0.044

Standard deviation......................... .... 0.065

Equation connecting variables:

$\text{Log } P = -3 \text{ Log } V + 3 \text{ Log } 3.011 \pm 0.044$

$dp/dv = -3$

cm. of mercury. At low pressures this product grew progressively smaller. This latter phenomenon was accounted for on the theoretical deduction that an internal pressure must exist as a result of the electrostatic attraction between the colloidal ion or particle and the dissociated diffusible ions. This electrostatic attraction opposes the osmotic imbibition, which otherwise would pro-

TABLE 115

Volume of water retained per gram of air dried Ca-saturated bentonite against various vacuum pressures

PRESSURE	WATER RETAINED PER GRAM BASIS 110°C.	(VOLUME) (PRESSURE$^{1/3}$)
cm. Hg	*cc.*	
22	1.051	2.94
22	1.066	2.99
20	1.149	3.118
20	1.352	3.081
15	1.312	3.235
15	1.350	3.329
10	1.433	3.087
10	1.363	2.936
5	1.492	2.551
5	1.533	2.621
Gravity	2.218	

Arithmetic mean.. 2.989 ±0.157
Standard deviation.. 0.233
Equation connecting variables:

$$\text{Log } P = -3 \text{ Log } V + 3 \text{ Log } 2.989 \pm 0.157$$

$$dp/dv = -3$$

TABLE 116

Volume of water retained per gram of air dried K-saturated bentonite against various pressures

PRESSURE	WATER RETAINED PER GRAM BASIS 110°C.	(VOLUME) (PRESSURE$^{1/3}$)
cm. Hg	*cc.*	
30	1.814	5.639
40	1.554	5.315
40	1.59	5.438
50	1.435	5.288
50	1.398	5.152
60	1.355	5.307
70	1.288	5.309
70	1.231	5.073

Arithmetic mean.. 5.315 ±0.108
Standard deviation.. 0.16
Equation connecting variables:

$$\text{Log } P = -3 \text{ Log } V + 3 \text{ Log } 5.315 \pm 0.108$$

$$dp/dv = -3$$

ceed indefinitely or until the dissociated ions distributed themselves equally throughout the system and became separated from the micelle. This would be contrary to the principle of electroneutrality. At low pressures and under the conditions of gravity alone the gel must therefore retain less water than the quantity corresponding to the relationship existing at higher pressures.

The experiments herein described showed that the same definite relationship exists independent of the nature of the cation in combination with the colloid, but that the constants obtained for each ion may vary considerably.

The untreated bentonite yields a constant the arithmetic mean of which is 13.54 ±0.181. This is considerably greater than the value (10.70) found by Mattson in his original experiments and must chiefly be ascribed to the fact that the bentonite here employed was freed from sand and silt.

TABLE 117

Volume of water retained per gram of air-dried Li-saturated bentonite against various vacuum pressures

PRESSURE	WATER RETAINED PER GRAM BASIS 110°C.	(VOLUME) (PRESSURE$^{1/3}$)
cm. Hg	*cc.*	
65	2.57	10.35
60	2.66	10.41
50	2.89	10.66
50	2.95	10.88
40	3.06	10.46
30	3.38	10.51
20	3.83	10.40

Arithmetic mean.. 10.54 ±0.117

Standard deviation... 0.173

Equation connecting variables:

$\text{Log } P = -3 \text{ Log } V + 3 \text{ Log } 10.54 \pm 0.044$

$dp/dv = -3$

The electrodialyzed (H-saturated) bentonite was studied first by dispersing the electrodialyzed gel in water without previous drying and then by dispersing the air-dried material after passing it through a 100-mesh sieve. In the first instance a constant of 7.5 was obtained as compared to 3.021 ±0.116 in the second case. The comparison is interesting, for it shows that the dispersion and imbibition must begin in the dry condition of the colloid if the influence of the various ions is to be studied. The electrodialyzed bentonite has inherited some of the high degree of dispersion of the original material and retains this, for its new condition abnormal, dispersion as long as it remains in the moist condition. The cohesive forces cannot then come into play and the dispersion is therefore not wholly reversible in the moist condition. As a result of adding $CaCl_2$, HCl, etc. to a highly swelled Na-saturated colloid no visible shrinkage can be observed. Only after the material has been dried does it become evident upon rewetting that it has lost its power to swell. So also in the present experiment. It is only in the dried material that the power of the exchangeable cations to overcome (by their dissociation and micelle formation) the cohesive forces expresses itself in its true proportion. (The importance of this principle in relation to the reclamation of alkali land is readily recognized.) The constant 3.021 may, from what has been said, be taken to represent the true influence of the H ions.

The Mg- and Ca-saturated bentonites yield constants which do not differ much from that of the electrodialyzed material. The values are for Mg 3.011 ±0.044 and for Ca 2.989 ±0.157. These gels have a tendency to crack at low water content. The relationship, therefore, can not be studied at high pressures by the method used. The difficulty might be overcome by the use of filter cylinders of steel and provided with an air-tight piston.

As in the case of the swelling (3, VIII) the K-saturated bentonite yields a value intermediate between that of the untreated bentonite (or Na-saturated) and the Ca-, Mg-, or H-saturated. The constant for K is 5.315 ±0.0108.

The constant for lithium is 10.54 ±0 117. Why this is so much lower than the constant of the original bentonite is difficult to say. We should have expected a higher value for this cation. It is possible that the drying of the electrodialyzed material caused some irreversible aggregation. It might also be due to the impurity of the LiOH, which contained considerable carbonate and may therefore not have fully saturated the colloid but left a Li-H-saturated product.

The influence of free electrolytes

In the preceding experiments the force with which water is retained is assumed to depend upon the degree of dissociation of the exchangeable cations. The experiments were performed in the absence of free electrolytes, which means that there was no suppression of the swelling according to the Donnan equilibrium. If the force with which water is retained by the colloid is the result of a higher osmotic pressure within the micellar solution, then the power of water retention should be suppressed by the presence of electrolytes, and this suppression should be governed by the concentration and by the valence of the ions. In the case of a negative colloid a chloride should suppress the osmotic pressure, and hence the water retaining power, more than a sulfate in equivalent concentrations. The higher the valence of the ions of the same sign of charge as the colloid, the smaller, according to the Donnan equilibrium, the suppression (3, I). The higher the valence of the ions of opposite sign of charge, in this case the cations, the greater the suppression. But we cannot compare the suppressing effect of, let us say, Na and Ca ions of a free electrolyte because superimposed on this difference in suppression will be the much greater difference in suppression resulting from the cation exchange. In one case we will have a highly dissociated Na-saturated colloid, in the other a slightly dissociated Ca-saturated colloid. We must work with a common cation (in the case of a negative colloid) and are therefore able to compare only the valence effect of the anions.

The effect of 0.5 N solutions of NaCl and Na_2SO_4 is shown in tables 118 and 119.

TABLE 118

Volume of solution retained per gram of air-dried electrodialyzed bentonite against various vacuum pressures when 0.5N (Na_2SO_4) is used as a dispersion medium

PRESSURE	SOLUTION RETAINED PER GRAM BASIS 110°C.	(VOLUME) ($PRESSURE^{1/3}$)
cm. Hg	*cc.*	
10	1.04	2.23
10	1.07	2.30
10	1.06	2.29
15	0.920	2.274
15	0.937	2.316
15	0.911	2.251
20	0.878	2.38
30	0.775	2.41
30	0.798	2.48

Arithmetic mean.. 2.325 ±0.052

Standard deviation.. 0.0769

Equation connecting variables:

$\log P = -3 \log V + 3 \log 2.3257 \pm 0.0519$

$dp/dv = -3$

TABLE 119

Volume of solution retained per gram of air-dried electrodialyzed bentonite against various vacuum pressures when 0.5N NaCl is used as a dispersion medium

PRESSURE	SOLUTION RETAINED PER GRAM BASIS 110°C.	(VOLUME) ($PRESSURE^{1/3}$)
cm. Hg	*cc.*	
10	0.837	1.80
10	0.845	1.82
10	0.839	1.80
15	0.770	1.902
15	0.765	1.889
15	0.759	1.876
20	0.666	1.81
20	0.726	1.97
30	0.630	1.96
30	0.625	1.95
30	0.636	1.97

Arithmetic mean.. 1.886 ±0.043

Standard deviation.. 0.0672

Equation connecting variables:

$\log P = -3 \log V + 3 \log 1.886 \pm .0433$

$dp/dv = -3$

It will be noted (*a*) that the relationship between the pressure and water content is the same as in the experiments with pure water; (*b*) that the amount of water retained against the various pressures is markedly reduced by the presence of the salts; and (*c*) that the suppressing effect of the chloride is greater than that of the sulfate. The constant in the chloride solution is 1.886 ±0.043 whereas in the sulfate it is 2.325 ±0.052. This is in agreement with the osmotic equation of the Donnan equilibrium and points to an osmotic mechanism of the phenomenon of imbibition (3, I).

DISCUSSION

Whether the established relationship between the pressure and water content of the gel holds at very high pressures has not yet been determined. Deviations will doubtless be found at some point at high pressures, for at low water contents the remaining water will partly be held under the influence of the far greater forces of molecular attraction which are manifest by the heat of wetting and by the volume contraction. Then account must be taken of the irreducible space occupied by the solid particles and their interstices when the former are in contact with one another. We must further consider the water of hydration of the exchangeable cations. To what extent this water can be removed by pressure we do not know. It is obvious that the dissociated ions cannot be removed with the liquid, for then the gel would develop a powerful charge.

It might be objected that if the relationship should hold up to moderately high pressures the latter would soon be too high to be accounted for as an osmotic phenomenon. The exchangeable cations would not, unless completely dissociated, suffice to account for the pressures. But it is possible that the osmotic forces may be anomalous and indeed very much greater in a layer of the solution where there is an excess of one kind of ions, that is, in an electrically charged atmosphere of ions. Powerful electrostatic attractions, not present in normal electroneutral solutions, may cause a greater imbibition of water than corresponds to the actual concentration of the ions. The process would then be electrosmotic and not merely osmotic.

Whether the forces responsible for the phenomena of imbibition and swelling of soil colloids be anomalous osmotic forces or not, and whether or not there are in addition other unknown forces at play must be left undecided, but it must be admitted that the relationships previously established and enumerated in the first part of this paper together with the results of this investigation all point to an osmotic mechanism.

SUMMARY

The relationship between the applied pressure and the water content of bentonite gel saturated with various cations has been studied. The product $V. \sqrt[3]{P}$ (V = cc. water per gram dry bentonite and P = applied pressure) is a constant whose magnitude depends on the nature of the exchangeable cation. Half-normal NaCl instead of water yields a lower constant than 0.5

N Na_2SO_4. This is according to the osmotic equation of the Donnan equilibrium. An osmotic interpretation is given.

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WATER CONTENT OF SOIL COLLOIDS AS RELATED TO THEIR CHEMICAL COMPOSITION[1]

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A study of the water content of soil colloidal material may be divided into three different phases:

1. Water that is constitutionally or chemically combined within the crystal lattice of hydrated alumino-silicates, or the hydrated oxides of aluminum, iron, and silicon. This type of water is called "combined water."
2. Water that is adsorbed on the surfaces or in the pores of the colloid by molecular attractive forces. In other words, that water which colloidal material will adsorb from an atmosphere containing water vapor. It is usually referred to as "hygroscopic water."
3. Water taken up by soil colloids when brought in contact with a water surface. Mattson (20) classifies this type of water into capillary and osmotically imbibed water.

There are several factors that affect the amount of water in soil colloids, such as the organic matter present, the amount and nature of exchangeable cations and the silica-sesquioxide ratio. Although several investigators have shown that these are important factors to be considered in determining the water content of colloids, little work has been done to show any definite interrelationships between them or to explain the nature of the phenomena.

It is the object of this paper to discuss the results of a study of the amount and nature of the water in soil colloids as related to their chemical composition. The effect of the amount and nature of exchangeable cations present and the silica and sequioxide content will be treated in their relationship to combined and hygroscopic water. Water absorption determinations in the presence of a free water surface are not included. This study includes the separation and preparation of colloidal material of widely varying chemical composition and the determination of the following values: (*a*) SiO_2, Al_2O_3, and Fe_2O_3; (*b*) organic matter; (*c*) hygroscopicity over 3.3 per cent and 30 per cent H_2SO_4, and and (*d*) water loss as a function of temperature These determinations were made on the natural, untreated colloids, on colloidal material which had been mildly oxidized with an H_2O_2 treatment, and on material which had been alternately extracted with 0.1 *N* HCl and 2 per cent Na_2CO_3.

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REVIEW OF LITERATURE

The chemically combined water is usually considered as that part of the total water which is combined with the colloidal complex. It is designated as that water not removed at 110°C. but which is driven off at ignition; it is calculated as the difference between the loss on ignition and the organic matter present. There appears to be only a limited amount of data in the literature on this important phase of water retention. Data of Robinson and Holmes (23) and Holmes and Edgington (14) show that the combined water varies inversely with the silica-sesquioxide ratio. They attribute this to the relation between the combined water and the aluminum and iron oxides present. Harrassowitz (11) uses the amount of combined water in soils to determine the presence of free Al_2O_3. If the water content exceeds 13.92 per cent, which is the amount contained in kaolin, free Al_2O_3 or SiO_2 is considered to be present. Kiu-hie, Kobaijashi, et al. (18), in determining the water content of different colloids by means of a thermo-balance, find that the heating curves of Japanese acid clays, fuller's earth, and Florida earth are similar, and that the main part of the gel water is evolved before 170°C. The breaks in the curves are at 200°–400°, and the residual water is driven off up to 700°C.

The hygroscopic water is held on the surface of the particles by molecular attractive forces. The attraction of water by these forces takes place with the evolution of heat; this is known as the heat of wetting of colloids. Rodewald (24) and Mitscherlich (21) have shown that soils manifest no heat of wetting when the original moisture content is equal to their hygroscopicity. From a theoretical standpoint, therefore, wetting of a dry surface takes place in two stages: (*a*) movement of water perpendicular to the surface as brought about by the attractive power of the solid substance and (*b*) movement of water more or less parallel to the surface as produced by surface tension and other forces. It so happens in this particular study that we are concerned primarily with the first type of adsorption.

The hygroscopicity of colloidal material is determined by exposing the colloid to an atmosphere of known relative humidity. Two different values of humidity are commonly used in this country, namely, a moist atmosphere in equilibrium with a 3.3 per cent by weight H_2SO_4 solution and a drier atmosphere, which is in equilibrium with 30 per cent H_2SO_4. Hygroscopicity measurements in Europe are generally made over a 10 per cent H_2SO_4 solution.

Anderson (1), Anderson and Byers (2), Anderson and Mattson (3), and others have observed a general relationship between the SiO_2-sesquioxide ratio of the colloidal material and the amount of water adsorbed over 30 per cent H_2SO_4. Hygroscopicity increased with an increase in this ratio. Anderson and Byers found that the hygroscopicity over 3.3 per cent H_2SO_4 increased with an increasing SiO_2-sesquioxide ratio, but the relation was not so marked as that over the 30 per cent H_2SO_4.

The nature of the exchangeable cations on the colloidal exchange complex affects the hygroscopicity over 30 per cent H_2SO_4 quite significantly. Baver (4), working with different soils saturated with various cations, found that the K ion caused a distinct lowering of the hygroscopic coefficient; the Na ions exhibited a similar but not so significant effect; the H, Ca, and Mg ions increased the hygroscopicity, although the differences among the three were not large. Data for the hygroscopicity over 3.3 per cent H_2SO_4 showed similar relationships, but the effect of the cations was less pronounced. Thomas (25), in studying water adsorption of different colloids at various vapor pressures, observed that at low humidities the hygroscopicity of different soils followed the order: $Ca > H > Na > K$. At high relative humidities this order was: $Na > Ca > H > K$. The differences between the H- and Ca-soils, however, were not very great. In some instances at the higher humidities the H-soil adsorbed more water than the Ca-soil. Thomas explains the change in water adsorption by Na- and Ca-soils with vapor pressure as being due to the formation of hydrates. Anderson (1) studied the effect of the exchangeable cations on the water adsorption by the extracted soil colloids and reports similar relationships. Data from the Wellcome Tropical Research Laboratories (22) show the following relationships: for 50 per cent H_2SO_4, $H > Ca > Na > K$ and for 20 per cent H_2SO_4, $Na > H > Ca > K$. The higher value of Na-clays as compared with K-clays is usually attributed to differences in the hydration of these two ions. Perhaps the most suitable explanation that has as yet been offered for explaining the low hygroscopicity of K-systems as compared with Ca-, Mg-, and H-systems, is that of Jenny (16), who suggests that the size of the ion is the contributing factor.

Organic matter increases the hygroscopicity of soils and soil colloidal materials very markedly. Baver (6), styding four different soils that had been treated with hydrogen peroxide, found that the adsorption of water over 3.3 per cent H_2SO_4 due to organic matter varied from 20 per cent to 40 per cent. Anderson and Byers (2) observed that the presence of organic matter increased the hygroscopicity over 30 per cent H_2SO_4. The oxidation of the organic matter with H_2O_2 in a colloid containing 68.1 per cent organic matter reduced the hygroscopicity 75 per cent. Another colloid containing 61.5 per cent organic matter had its hygroscopicity reduced 40 per cent by oxidation. Organic soil colloids have a high surface activity and consequently great attractive powers for water molecules.

Little work has been done on the effect of the removal of the free oxides of Si, Al, and Fe on the properties of soil colloids. Mattson (19) treated several soil colloidal materials with a hot, saturated solution of aluminum chloride to peptize any precipitated aluminum and iron hydroxides. He found, for example, that the exchange capacity of the Norfolk colloid increased as a result of this peptization from 20.7 to 42.5 m.e. per 100 gm. of colloid. In other words, the surface activity of the colloidal material was more than doubled. Kerr (17), in attempting to isolate the alumino silicate active in base exchange and soil acidity, treated the soil alternately with 0.05 *N* HCl and 10 per cent Na_2CO_3 and found that the base exchange capacity of the residue was not greatly affected. Truog and Chucka (26) have attempted to purify colloidal systems by alternate extractions with 0.1 *N* HCl and 2 per cent Na_2CO_3 with fairly good results. Baver and Scarseth (8), in studying the profile of the Susquehanna fine sandy loam soil from Alabama, treated the colloidal material from each horizon alternately with 0.1 *N* HCL and 2 per cent Na_2CO_3 at 60°C.; extraction was continued until no precipitate of Si or Al was found in the filtrate. They found that the Si, Al, and Fe were removed in the same ratio as they occurred in the colloid, indicating the absence of free oxides in this extracted colloid.

These data strongly suggest the necessity of further study on the effect of free oxides on the colloidal properties of soils. This is especially true in those studies involving moisture determininations where the presence of free hydrated oxides of Al, Fe, or Si would be expected to increase the amount of combined water per unit weight of extracted colloid.

EXPERIMENTAL PROCEDURE

Colloidal material extracted from different soils and varying widely with respect to chemical composition and exchangeable cations was used in this investigation. The clay colloids were supplemented with systems of H-bentonite and H-permutite, which represent extreme variations in chemical composition. The colloidal clays containing different exchangeable cations were prepared by the addition of the hydroxide of the desired cation to the electrodialyzed colloidal material from the Putnam silt loam (subsoil) (5). These clays had been used in previous investigations in this laboratory.

The colloidal material prepared in this investigation was isolated from four soils according to the method of Bradfield (8). The following soils were used: (*a*) Lufkin clay (surface) from Alabama, (*b*) Putnam silt loam (subsoil) from Missouri, (*c*) Susquehanna clay (subsurface) from Alabama, and (*d*) Cecil clay (subsurface) from Alabama. The colloidal material from each soil was divided into three portions and treated as follows: (*a*) no treatment, (*b*) organic matter oxidized with a 6 per cent H_2O_2 solution, and (*c*) alternate extractions with 0.1

N HCl and 2 per cent Na_2CO_3 according to the method of Truog and Chucka (26). The analyses of SiO_2, Al_2O_3, Fe_2O_3, and P_2O_5 were made according to standard analytical methods.

The hygroscopicity was determined over 3.3 per cent H_2SO_4 and over 30 per cent H_2SO_4 by the usual method, except for the procedure of drying. After the samples had been exposed over the acid solutions and the moist weights obtained, the samples were dried in an ordinary oven at a series of different temperatures; namely, 60°, 85°, 110°, and 150°C. The weights of the samples were obtained after the samples had come to equilibrium at each temperature. The percentage of moisture lost between each two successive temperatures was calculated and the hygroscopicity was taken as that percentage lost at 110°.

The chemically combined water was determined as follows: The electric furnace of a carbon combustion train was calibrated by connecting resistances in series with it so that the temperatures of 110°, 150°, 200°, 300°, 400°, 500°, 600°, and 800°C. could be obtained. The temperatures were determined by means of a pyrometer. The furnace was then connected on the inlet end with a tower containing anhydrous $CaCl_2$, and this in turn was connected with a tube containing ascarite. The outlet end of the furnace was connected with anhydrous $CaCl_2$ bulbs and an ascarite bulb, all of which were connected in series, with the ascarite bulb last. This set-up was then connected to a tank of oxygen gas, so that a slow steady flow of the gas through the apparatus could be maintained. The gas entering the furnace thus was freed from CO_2 and water vapor. A sample of colloidal material, which had been previously dried at some particular temperature and whose weight was accurately known, was then placed in the furnace which was set at the desired temperature. An alundun boat was used to hold the sample. The sample and the boat were weighed by placing them in a 6-inch glass tube, which could be tightly closed by means of a screw top. The weights of the $CaCl_2$ and ascarite bulbs having been obtained, the flow of oxygen was started through the apparatus and continued until no more moisture was given off by the sample. This point was determined by weighing the $CaCl_2$ bulbs at intervals until a nearly constant weight was obtained. It was found that for the temperatures up to 400°C. this equilibrium point was reached in 6 to 8 hours; for the remaining temperatures a shorter time of about 5 hours was necessary. When this equilibrium point was reached, the $CaCl_2$ and ascarite bulbs were accurately weighed. The increase in weight of the $CaCl_2$ bulbs and the ascarite bulb gave the amounts of water and CO_2 given off by the sample. The temperature was then raised to the next higher point and the aforementioned procedure was repeated until the sample was ignited at 800°C. The sample remained in the furnace throughout the procedure. The weight of the sample was obtained at the finish in order that its ignition weight might be obtained. The $CaCl_2$ in the bulbs was replaced by freshly dehydrated $CaCl_2$ after three or four samples had been analyzed.

When the colloidal material was heated at the oxidizing temperatures, part of the water taken up by the $CaCl_2$ bulbs came from the colloidal material itself and part from the oxidation of the organic matter present. In order to make corrections for this, it was assumed that the carbon and hydrogen existed in the organic matter in the ratio of one carbon atom to two hydrogen atoms. Thus, according to this assumption, during the oxidation of the organic matter, equal molecular weights of CO_2 and water are produced. In the calculation of the amount of water removed from the colloidal material itself at any particular temperature, an amount of water equal in molecular value to the CO_2 evolved is subtracted from the total amount of water absorbed by the $CaCl_2$ bulbs. It was found that the amount of water given off by the sample according to this method of calculation agreed within 2 per cent of the actual ignition loss of the sample, when the correction for the loss in weight due to the oxidation of the organic matter was applied Data obtained with this method of calculation agreed very well with those obtained by the use of the conventional factor, $CO_2 \times 0.471$.

RESULTS

Chemical composition of colloidal materials

The chemical analyses of the colloidal material prepared in this investigation are given in table 1. These data show that the four types of colloidal material vary widely in their chemical compositions The amount of organic matter removed by the H_2O_2 treatment varied from 77 per cent for the Putnam to 55 per cent for the Susquehanna colloid. It is interesting to note that the amount of organic matter present in the untreated colloids is comparatively low. The mild oxidizing treatment had no appreciable effect on the inorganic fraction since the SiO_2 - R_2O_3 ratios were not affected. This confirms previous investigations of Baver (7) on the nature of the exchange complex.

The extraction with 0.1 *N* HCl and 2 per cent Na_2SO_3 produced a marked change in the composition of Putnam colloid. The SiO_2-R_2O_3 and SiO_2-Al_2O_3 ratios of the Putnam were materially increased by the extraction. This treatment caused a slight increase in these ratios in the Lufkin colloid. There were no significant effects on the Susquehanna and Cecil colloids. It is interesting to note, however, the analyses of the material extracted from these colloidal clays. These results are given in table 2. The amount of oxides removed from each colloid varied considerably; the Putnam colloid lost 21.2 per cent on extraction, the Lufkin 10.8 per cent, the Cecil 5.8 per cent, and the Susquehanna 5.4 per cent. The large amount of extracted oxides in the Putnam colloid indicates that it contains considerable quantities of gel-like material. Much smaller amounts of such material exist in the other three colloids. These differences should be expected when the nature of the soil is considered. The Putnam colloid represents material extracted from the heavy B-horizon of a soil presumably developed under a podzolic type of soil weathering. Consequently, since it is taken from the zone of accumulation it should contain a certain amount of hydrated oxides. The SiO_2-R_2O_3 ratio of the extracted material is considerably lower than that of the colloid, which indicates that free Al_2O_3 and Fe_2O_3 are present. If the large amount of SiO_2 removed has resulted from a breaking down of an alumino- or ferro-silicate, this colloidal clay complex must be relatively instable. In all probability, however, much of this silica is in the uncombined state.

TABLE 1

Chemical composition of the colloidal material

COLLOID	SiO_2	Al_2O_3	Fe_2O_3	P_2O_5	ORGANIC MATTER	IGNITION LOSS	$\frac{SiO_2}{R_2O_3}$	$\frac{SiO_2}{Al_2O_3}$
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Lufkin—untreated	58.29	20.98	7.76	0.04	0.80	8.00	3.81	4.71
Lufkin—oxidized	58.52	21.41	7.67	0.02	0.33	7.86	3.78	4.64
Lufkin—extracted	60.35	21.26	7.69	0.01	0.51	6.94	3.91	4.82
Putnam—untreated	52.40	24.24	8.90	0.04	1.73	10.95	2.97	3.67
Putnam—oxidized	53.42	24.81	9.03	0.02	0.39	9.72	2.97	3.65
Putnam—extracted	56.15	23.53	8.88	0.01	0.89	8.68	3.26	4.05
Susquehanna—untreated	45.01	26.22	12.77	0.15	0.95	11.34	2.22	2.91
Susquehanna—oxidized	45.78	26.95	12.90	0.04	0.43	10.71	2.21	2.88
Susquehanna—extracted	46.72	27.51	13.19	0.06	0.52	10.13	2.21	2.88
Cecil—untreated	34.72	33.71	13.30	0.13	1.81	16.01	1.40	1.75
Cecil—oxidized	35.35	34.51	13.19	0.08	0.71	14.82	1.40	1.74
Cecil—extracted	36.60	34.22	14.33	0.09	0.81	13.79	1.43	1.82

TABLE 2

SiO_2, Al_2O_3, and Fe_2O_3 removed from the colloidal material by extraction

COLLOID	SiO_2	Al_2O_3	Fe_2O_3	$\frac{SiO_2}{R_2O_3}$	$\frac{SiO_2}{Al_2O_3}$
	per cent	*per cent*	*per cent*		
Lufkin	6.4	3.4	1.0	2.74	3.31
Putnam	12.4	6.5	2.3	2.62	3.22
Susquehanna	2.9	2.1	0.4	2.01	2.29
Cecil	1.95	3.5	0.4	0.87	0.93

The Susquehanna and Cecil colloids have been weathered under lateritic influences. The former does not represent as late a stage in this type of weathering as the Cecil. Any free oxides in these colloids probably occur to a great extent in the crystalline form and, therefore, are more resistant to the dissolving action of HCl and Na_2CO_3. The material removed from the Cecil has a SiO_2-R_2O_3 ratio approximately one-half that of the untreated colloid. This suggests that the extracted material must come from a complex or compound that contains a high percentage of alumina. Since such a small amount was decomposed, however, this source of the Al_2O_3 must be very stable. It is possible that the colloidal material contains hydrargillite or similar compounds The SiO_2-R_2O_3 ratio of the Susquehanna colloid did not change very much. as a result of the extraction. This confirms previous data by Baver and Scarseth (8) on this particular clay. Evidently, the present colloidal complex is very stable. The material extracted from the Lufkin colloid contains much more Al_2O_3 and Fe_2O_3 in proportion to the SiO_2 than the original colloid. The sources of these oxides is very stable also, although the stability is not so great as in the case of the Susquehanna and Cecil.

TABLE 3

Hygroscopicity of various soil colloidal materials

COLLOID	HYGROSCOPICITY OVER	
	30 per cent H_2SO_4	3.3 per cent H_2SO_4
	per cent	*per cent*
H-bentonite	21.54	50.22
H-permutit	22.03	52.03
H-clay	18.13	44.76
Li-clay	17.13	56.12
Na-clay	16.53	49.22
K-clay	12.75	31.12
Mg-clay	17.08	39.32
Ca-clay	17.37	40.91
Ba-clay	16.29	41.95
Lufkin—untreated	20.02	42.06
Lufkin—oxidized	20.13	40.02
Lufkin—extracted	21.91	52.85
Putnam—untreated	17.40	36.50
Putnam—oxidized	17.82	35.59
Putnam—extracted	20.38	45.17
Susquehanna—untreated	15.07	36.98
Susquehanna—oxidized	15.54	34.56
Susquehanna—extracted	16.62	43.85
Cecil—untreated	5.74	37.15
Cecil—oxidized	6.10	37.80
Cecil—extracted	7.39	39.97

Water content of different colloidal materials

Permutite, bentonite, and clay. In order to determine any differences or similarities that may exist in the water content of various alumino-silicates, electrodialyzed samples of permutite, bentonite, and Putnam clay were studied.

By the use of the electrodialyzed material, the effect of the nature of the exchangeable cation was kept constant; that is, pure H-permutite, H-bentonite, and H-clay were used. Permutite was chosen as representing typical artificial alumino-silicates It has a SiO_2-R_2O_3 ratio of about 3.0, an open-packed structure, and a high exchange capacity. Bentonite is a typical natural alumino-silicate with a SiO_2-R_2O_3 ratio of 5.0, a more or less close-packed structure, and an exchange capacity of about 100 m.e. per 100 gm. of colloid. Several soil investigators attribute the colloidal properties of the soil to its bentonitic constituents. The colloidal Putnam clay has a SiO_2-R_2O_3 ratio of approximately 3.2, a fairly close-packed structure, and an exchange capacity of about 60 m.e. per 100 gm. of colloid.

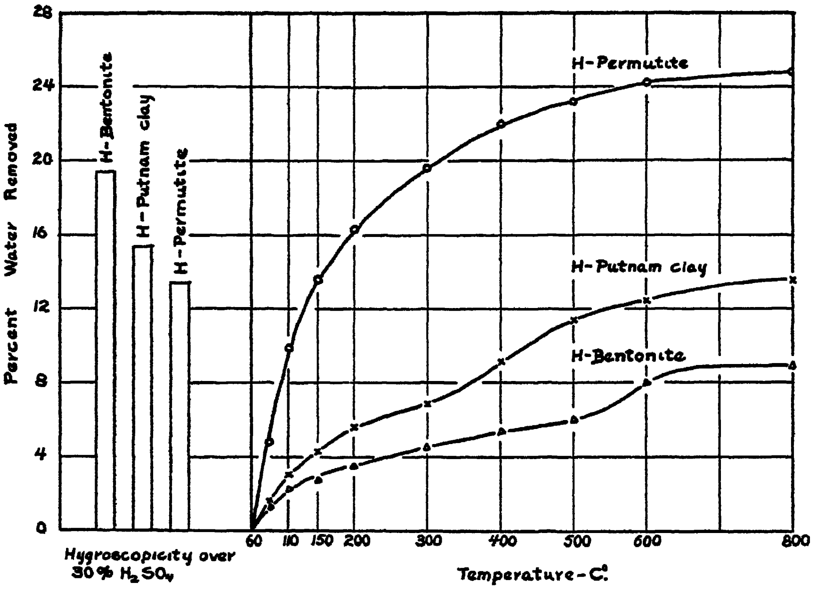

FIG. 1. LOSS OF WATER FROM COLLOIDAL MATERIAL AS A FUNCTION OF TEMPERATURE

The hygroscopicity of these colloids is given in table 3 and figure 1. The percentage of water removed from the colloid as a function of temperature is shown in figure 1 and in tables 4 and 6. The rate of removal of water as related to temperature is shown in figure 2. These data point out that bentonite has the highest hygroscopicity, and permutite the lowest. In other words bentonite apparently has the greatest surface activity as evidenced by a larger attractive force for water vapor molecules. On the other hand, permutite loses the most water from 110° to ignition and bentonite the least. Putnam clay occupies an intermediary position. It is interesting to note that the loss of water from the permutite is a relatively simple function of temperature. This is not true with bentonite and Putnam clay. These two colloids exhibit distinct breaks in the water loss curves. The rate of water removal from the Putnam clay gradually decreases to a minimum at about 275°C. where it

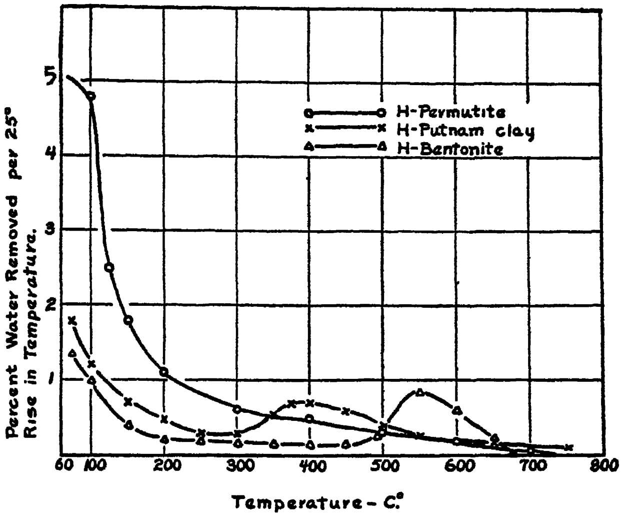

FIG. 2. RATE OF LOSS OF WATER FROM COLLOIDAL MATERIAL AS A FUNCTION OF TEMPERATURE

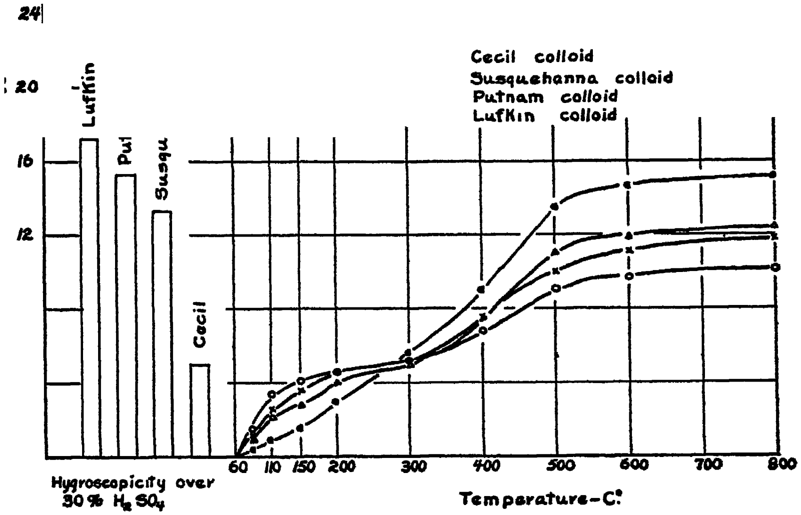

FIG. 3. LOSS OF WATER FROM DIFFERENT SOIL COLLOIDS AS A FUNCTION OF TEMPERATURE

increases to give a maximum point at 400°C. The bentonite curve shows a broad minimum range from 200° to 475°C. where it rises to a maximum point at 550°C. These maximum points in the rate of water loss curves may be interpreted as representing points at which water is being lost as a result of the breaking up of the crystal lattice of a hydrated constituent of the clay or bentonite. Additional experiments have shown that mixtures of bentonite and Putnam clay exhibit the maximum points of the clay as well as of the bentonite. Consequently, these maximum points are apparently characteristic for each colloid. The possibilities of their significance will be discussed later in this paper.

TABLE 4

Moisture removed from soil colloidal materials at different temperatures

COLLOID	MOISTURE REMOVED AT									
	60° to 85°	85° to 110°	110° to 150°	150° to 200°	200° to 300°	300° to 400°	400° to 500°	500° to 600°	600° to 800°	60° to 800°
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
H-bentonite	1.49	0.92	0.44	0.66	1.13	0.75	0.59	1.97	1.01	8.96
H-permutit	5.46	4.45	3.68	2.70	3.27	2.44	1.24	1.01	0.51	24.76
H-clay	1.93	1.18	1.21	1.38	1.13	2.40	2.14	1.00	0.98	13.35
Li-clay	1.95	1.61	0.90	0.77	0.97	2.61	2.56	1.19	0.35	12.91
Na-clay	1.09	0.73	0.90	0.77	0.65	2.61	2.36	0.78	0.50	10.39
K-clay	0.83	0.65	0.91	1.01	0.66	2.17	2.43	0.97	0.55	10.18
Mg-clay	2.14	1.47	1.29	1.32	0.91	2.56	2.12	1.33	0.59	13.73
Ca-clay	1.96	1.48	1.43	0.93	0.73	2.50	2.36	1.09	0.80	13.28
Ba-clay	2.03	1.11	1.03	0.91	0.77	2.35	2.47	0.79	0.49	11.95
25 per cent K-clay	1.41	1.22	0.98	1.13	1.07	2.24	2.26	1.02	0.61	11.94
50 per cent K-clay	1.22	1.11	0.96	1.09	0.71	2.34	2.24	1.17	0.63	11.47
75 per cent K-clay	0.97	0.77	0.68	1.08	0.79	2.71	1.91	1.11	0.73	10.75
Lufkin—U	1.77	1.65	0.65	0.54	0.59	1.60	2.26	0.62	0.42	10.10
Lufkin—O	2.01	1.58	0.69	0.72	0.60	1.75	1.94	0.68	0.51	10.48
Lufkin—E	1.40	0.76	0.70	0.56	0.75	2.19	1.28	0.58	0.40	8.62
Putnam—U*	1.40	1.17	1.05	0.95	0.59	2.47	2.36	1.11	0.70	11.80
Putnam—O†	1.60	1.28	0.85	0.78	0.81	2.36	2.48	1.10	0.57	11.83
Putmam—E‡	1.48	0.76	0.73	0.65	1.10	1.95	2.00	0.89	0.50	10.06
Susquehanna—U	1.15	1.05	0.61	1.16	1.00	2.66	3.33	0.88	0.63	12.47
Susquehanna—O	1.16	0.96	0.72	1.05	1.13	2.49	3.51	0.97	0.68	12.67
Susquehanna—E	1.14	0.67	0.66	0.90	1.13	2.23	3.55	0.78	0.34	11.40
Cecil—U	0.53	0.42	0.65	1.39	2.63	3.27	4.64	1.07	0.63	15.23
Cecil—O	0.56	0.46	0.60	1.71	2.20	2.98	5.35	0.62	0.84	15.32
Cecil—E	0.70	0.60	0.40	1.36	2.00	2.35	5.41	0.71	0.52	14.05

* Untreated colloid.

† Colloid oxidized with H_2O_2.

‡ Colloid alternately extracted with 0.1 *N* HCl and 2 per cent Na_2CO_3.

Various soil colloids. The water relationships of the colloidal material extracted from the different soils were also studied. The chemical composition of these colloids, as expressed by their SiO_2-R_2O_3 ratios, varied as follows: Lufkin with a ratio of 3.81, Putnam with a ratio of 2.97, Susquehanna with a ratio of 2.22, and Cecil with a ratio of 1.40. The water losses from these colloids as a function of temperature are shown in figure 3 and tables 4 and 6. Their hygroscopicities are also shown in figure 3. These data point out that hygroscopicity increases with the SiO_2-R_2O_3 ratio and that the combined water decreases as this ratio becomes larger. The hygroscopicity values for the Lufkin, Putnam, Susquehanna, and Cecil are 20.02, 17.40, 15.07, and 5.74 per cent, respectively; the corresponding amounts of combined water are 10.10, 11.80, 12.47 and 15.23 per cent.

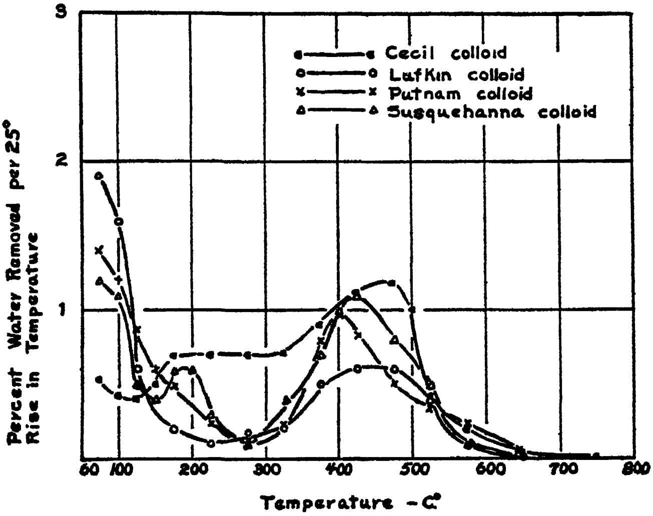

FIG. 4. RATE OF LOSS OF WATER FROM DIFFERENT SOIL COLLOIDS AS A FUNCTION OF TEMPERATURE

The rate of water loss from these colloids as a function of temperature is shown in figure 4. It is evident that these four colloidal materials do not contain the same constituents that contribute to the water loss at the various temperatures. The Cecil colloid curve exhibits a minimum point at about 125°C.; it then rises to give a constant rate of water loss between the temperatures of 175° to 325°; and, finally, reaches a maximum point at about 475° from which it rapidly declines to almost zero at 600°. The Susquehanna curve shows two maximum points, one at about 200° and the other at 425°; it also has two minimum points, one at 150° and the other at 275°. Apparently, in the Cecil and Susquehanna colloids there are at least two different constituents affecting the rate of water loss. These materials may or may not be the same in the two colloids. It is highly probable, however, that the substances responsible for the minimum points in the proximity of 150° are similar in both colloids; the same applies to the maximum point at about 450°. The curves for the Lufkin and Putnam colloids are very much alike, although not identically so. It seems logical to assume that there is a constituent common to all of these colloids that is responsible for the maximum point at temperatures between 400–500°C. A more detailed discussion of these maximum and minimum points will appear later in the paper.

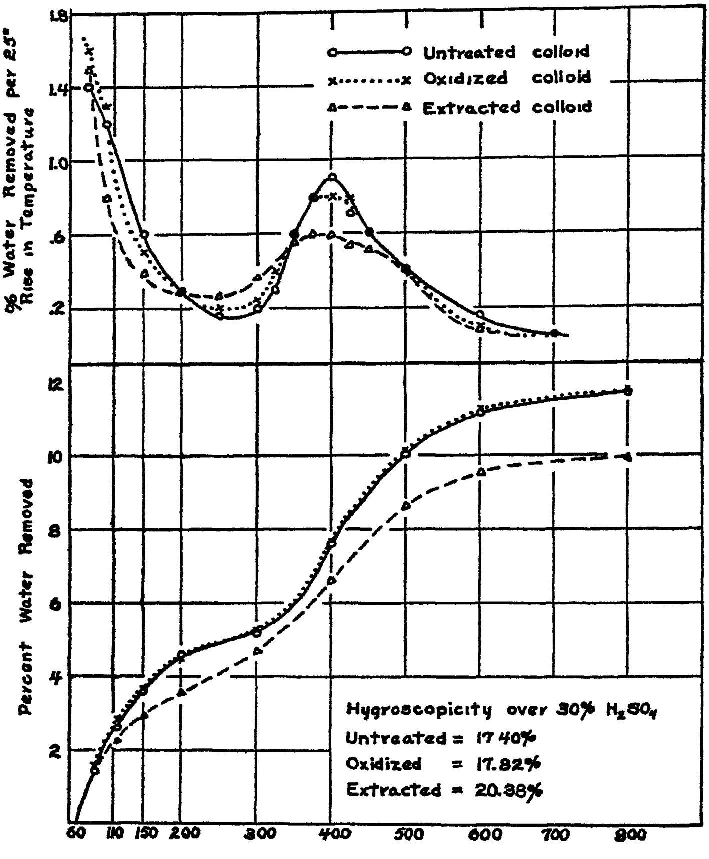

FIG. 5. LOSS OF WATER FROM THE PUTNAM COLLOID AS A FUNCTION OF TEMPERATURE

Factors affecting the water content of colloids

Organic matter and free oxides of Al, Fe, and Si. It should be expected that the water content of a given soil colloidal material would vary with the amount of organic matter present. This is especially true for the hygroscopicity values, as has been shown experimentally by Baver (6) and by Anderson and Byers (2). The small amount of organic matter in these particular colloids, however, does not appreciably affect the hygroscopicities as shown in table 3. The water loss curves for the Putnam colloid, with and without organic matter, are practically coincident as shown in figure 5. These curves should be the same, since the amount of water coming from the oxidation of the organic matter is corrected for in the data from which the curves are plotted. The curves for the Putnam colloid are typical for the other colloids.

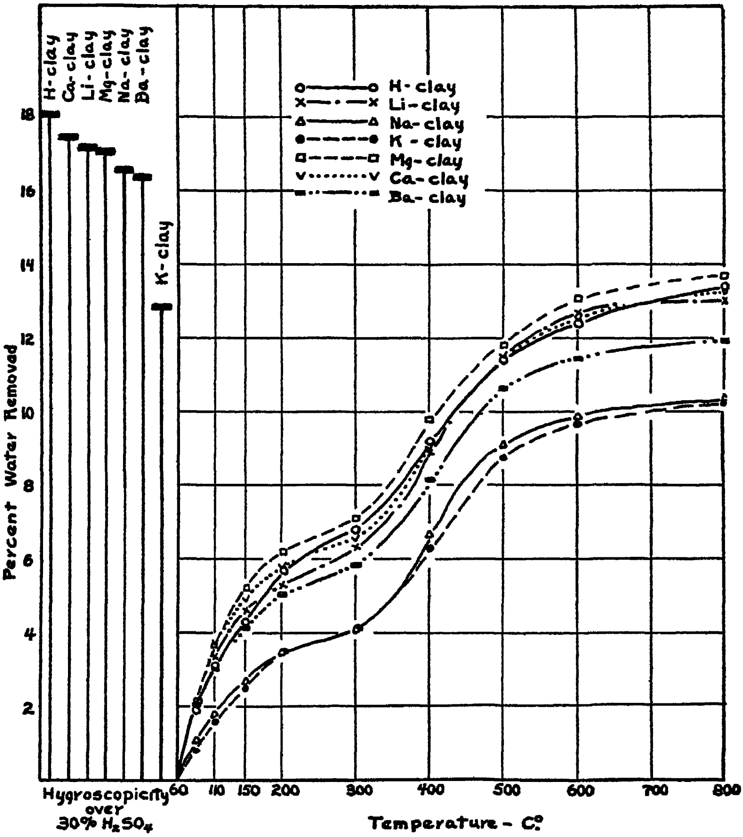

FIG. 6. LOSS OF WATER FROM THE PUTNAM COLLOID AS AFFECTED BY THE NATURE OF THE EXCHANGEABLE CATIONS

Extraction of the colloids with 0.1 *N* HCl and 2 per cent Na_2CO_3 increased the amount of water adsorbed by the colloid from an atmosphere of water vapor; this is especially true in the more humid atmospheres. The increased adsorption of water vapor after the extraction process, is probably due to the increased dispersion of the colloid. The removal of any gelatinous oxides which may have covered active adsorption points in the surface of the colloid would also increase the hygroscopicity values. The water loss curves for the extracted Putnam colloid have about the same shapes as the untreated samples.

There is a decrease in the amount of combined water, however, which indicates that part of the water loss is due to the dehydration of hydrated oxides of Al, Fe, and Si.

The nature of the exchangeable cations. The nature of the exchangeable cations has a pronounced effect upon the amount of water in a colloidal clay. The properties of the different cations which may affect the water content are: (*a*) hydration of the cation, (*b*) the ionic radius, and (*c*) the effect of the cation upon the dispersity of the colloidal system.

The Putnam colloid, saturated with different cations, was used in this particular part of the study. The data obtained are shown in tables 4 and 6 and in figures 6 and 7. It is seen that the presence of highly hydrated cations on the colloidal complex tends to increase the amount of water in the system. This effect is more apparent at lower temperatures and higher vapor pressures. The data show that the hygroscopicity over 30 per cent H_2SO_4 of the different clay salts increases according to the series:

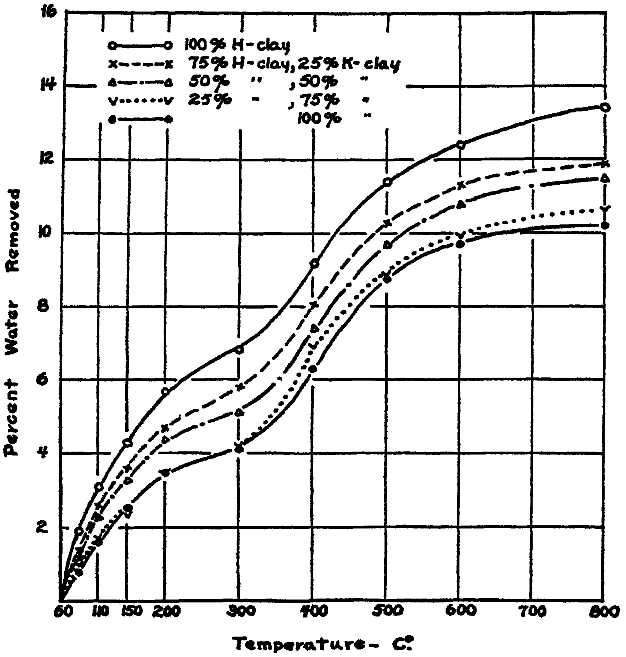

FIG. 7. RELATION BETWEEN THE EXCHANGEABLE HYDROGEN AND POTASSIUM IONS TO THE LOSS OF WATER FROM THE PUTNAM COLLOID

$$H > Ca > Li \gtreqless Mg > Na \gtreqless Ba > K$$

The effect of the nature of the cations is significant up to a temperature of about 300°. The water loss between 60° and 300° follows the order:

$$Mg > H > Ca > Li > Ba > Na \gtreqless K$$

The total water loss from the amount adsorbed over 30 per cent H_2SO_4 to that lost at 800° follows the order:

$$\mathrm{H} > \mathrm{Mg} = \mathrm{Ca} > \mathrm{Li} > \mathrm{Ba} \overset{=}{>} \mathrm{Na} > \mathrm{K}$$

The curves in figure 7 illustrate most vividly the decrease in the hydration of a colloidal clay as the H ion is gradually replaced by the K ion. Similar data have been obtained by Jenny (15) with wet permutites.

It is evident that the water loss from the colloids saturated with the monovalent cations follows according to their hydration with the possible exception of the H ion. The relative hydration of the H ion is not exactly known, although on the basis of transference measurements it is usually considered as not being hydrated. The divalent clays tend to be more hydrated as the hydration of the ion increases. It is difficult to explain, however, on the basis of pure hydration, why the divalent systems, Mg- and Ca-clays, contain more water than Li- and Na-clays. The fact that the H-system is the most highly hydrated is even more difficult to understand.

The concept of Jenny (16), in which he considers the relative size of the ion as contributing an important part in the hydration of alumino-silicates, offers a means of understanding these data. Jenny considers, especially for permutites, that there are large cavities present "which are filled with adsorbed (wandering) ions and water molecules. For a given crystal lattice the number of water molecules which can be packed into one cavity apparently depends upon the number and size of the exchangeable ions within the cavity. *The smaller the number and the true (crystal lattice radii) size of the adsorbed ions, the greater will be the number of water molecules in the system.*" He applies this concept to permutites with good results. It is apparent that such a concept could be possible in a permutite which has a porous structure. The question arises whether it will correlate with the facts in colloidal clay systems.

According to the Jenny hypothesis, the different clays should be hydrated according to the following scheme:

$$\mathrm{H} \left\rangle \begin{array}{ccccc} \mathrm{Li} & > & \mathrm{Na} & > & \mathrm{K} \\ \wedge & & \wedge & & \wedge \\ \mathrm{Mg} & > & \mathrm{Ca} & > & \mathrm{Ba} \end{array} \right.$$

In other words, H-systems should be the most highly hydrated because of the small size of the H ion and, consequently, because of a larger cavity volume accessible to water molecules. Likewise, within the monovalent or divalent cation groups, hydration would increase with decreasing size of the ions. Since Mg and Li ions are of the same size, Mg-systems should be the more highly hydrated because there would be only one-half as many ions present in the Mg- as in the Li-systems. Similarly, Ca-systems should be more hydrated than the Na-, and Ba-systems should contain more water than the K- saturated systems. The results obtained in this study show that the concept of Jenny holds rather rigidly for clays as well as for permutites.

Jenny does not attempt to arrange the various cations in a continuous series relative to their effects upon the hydration of alumino-silicates. Although he has orginated a valuable relationship between certain divalent and monovalent cations, it would be of utmost importance if the different cations could be placed in one continuous series. This shall be attempted for the first time on the basis of the following hypothetical reasoning:

It might be expected that the effective volume of two monovalent ions would be greater than that of one divalent ion which has the same actual ionic volume as the two monovalent. When two ions touch they would thereby occupy a space somewhat larger than the sum of their own volumes; this volume would not be occupied by water molecules. One might assume, therefore, that when two ions touch each other they would occupy an effective volume similar to a cylinder, the radius of which would be that of the ion and the height of which would be the sum of the diameters of the two ions. This picture is taken as a working hypothesis, with the realization that the effective ionic volume would not necessarily have such a simple shape. Consequently, a clay saturated with divalent cations should contain slightly more water than one saturated with monovalent cations in the case where both cations have approximately the same ionic volume per 60 m.e.

TABLE 5

Ionic radius and volume of several exchangeable cations

	H	Li	Na	K	Mg	Ca	Ba
Radius in Å (Goldschmidt, Pauling)	0.45	0.78	0.98	1.33	0.78	1.06	1.45
Volume of ion ($\times 10^{24}$ cc.)	0.38	1.99	3.94	9.85	1.99	4.99	12.80
Volume of 60 m.e. of ions ($\times 10^{3}$ cc.)	1.38	7.24	14.33	35.81	3.62	9.09	23.27
Relative volume* of ions (H = 1)	1.00	5.23	10.38	25.94	1.75	4.39	11.24

* Volume of monovalent cations considered as a cylinder formed by two ions touching each other.

The data in table 5 give the ionic radii, ionic volume, volume of 60 m.e. of ions, and the relative volume occupied by the exchangeable ions in the colloid when the effective volume of the H ion is considered as 1.0, These data suggest, in lieu of the aforementioned reasoning, that the Ca-clay would be more highly hydrated than the Li-clay even though the true ionic volume of the Ca ions in the Ca-clay is larger. Likewise, although the true ionic volume of the Ba-ions is much larger than that of the Na ions in the Ba- and Na-clays, respectively, the effective volume of the Na ions is almost as large as that of the Ba. Consequently, Ba-clay should be almost as highly hydrated as the Na-clay. Since the values of the effective volumes for the Ba and Na ions are so close together when using the "cylinder" concept as a basis of calculation, the absolute effective volume of the Na ions probably is even larger than for the Ba. If this is true the Ba-clay would be more highly hydrated than the Na. On the basis of effective ionic volumes the theoretical cation series for the hydration of clays would then be:

$$H > Mg > Ca > Li > Ba \gtreqless Na > K$$

The data in this study show that this series holds for the water loss between 60° and 800°C. with the exception of a reversal of the Mg and H ion positions. The amount of water loss from that amount adsorbed over 30 per cent H_2SO_4 to that driven off at 800° follows the theoretical series. The amount of water adsorbed over 30 per cent H_2SO_4 also follows the theoretical order except that Mg and Ca are about the same.

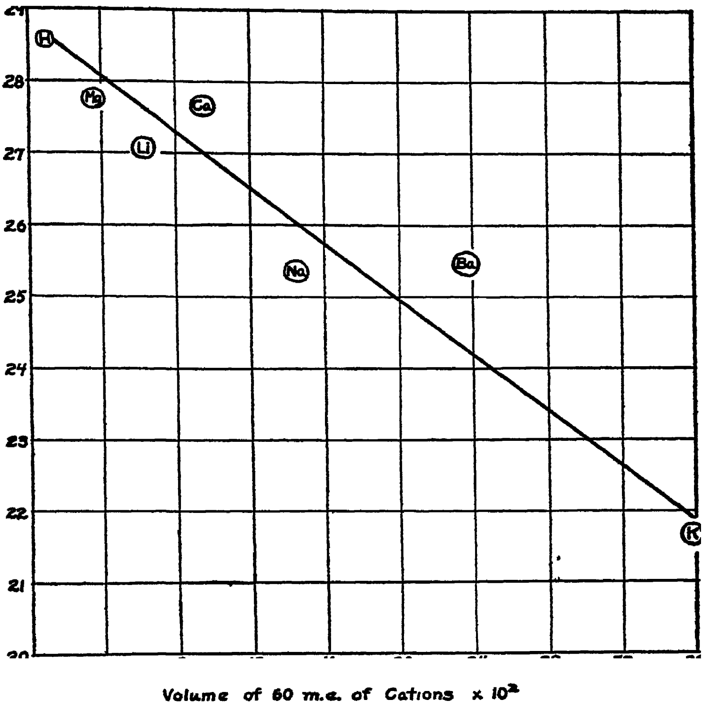

FIG. 8. RELATION OF THE VOLUME OF THE EXCHANGEABLE CATIONS TO THE WATER ADSORPTION OF THE PUTNAM COLLOID

These relationships are graphically illustrated in figure 8. The data show that the water content of the various clay systems is inversely proportional to the volume occupied by the exchangeable cations. The results of this study point out very strongly that the hydration of Putnam clay is a function of the true volume of the ions. Further studies with different clays are being made in order to determine whether the suggested cation series for the hydration of clays will hold rigidly under all conditions. This is a step to coördinate the present knowledge of monovalent and divalent cation behavior.

The nature of the curves in figure 6 above 300°C. indicates that the exchangeable cations have little or no effect upon the water lost at these temperatures. The Ba, Na, and K ions show a tendency to decrease the amount of water given off above 300°, but it is doubtful whether these decreases are significant or not. This strongly suggests that the water lost by the colloidal material above 300° is only that water which is chemically combined with the colloidal complex.

TABLE 6

Intensity of water retention by colloidal material at different temperature and humidity ranges

COLLOID†	(*X* TO *Y*)*	*X* TO 60°	60°–110°	110°–800°	60° TO MIN-POINT	MIN-POINT TO 800°	*X* TO 800°
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
H—bent	28.68	19.40	2.14	6.82	5.59	3.37	28.36
H—perm	30.00	13.36	8.67	16.09			38.12
H—clay	26.63	15.44	2.69	10.66	6.27	7.09	28.79
Li—clay	38.99	14.14	2.99	9.92	5.72	6.67	27.05
Na—clay	32.69	14.97	1.56	8.83	3.82	6.58	25.36
K—clay	18.37	11.50	1.25	8.93	3.72	6.46	21.68
Mg—clay	22.24	13.99	3.09	10.64	6.68	7.04	27.72
Ca—clay	23.54	14.44	2.93	10.34	6.17	7.12	27.71
Ba—clay	25.66	13.54	2.75	9.20	5.47	6.47	25.49
Lufkin—U	22.03	17.14	2.88	7.20	4.94	5.14	27.22
Lufkin—O	19.89	17.03	3.10	7.40	5.29	5.21	27.53
Lufkin—E	30.94	20.00	1.91	6.71	3.63	4.99	28.62
Putnam—U	19.10	15.24	2.16	9.64	4.94	6.87	27.04
Putnam—O	17.77	15.38	2.44	9.40	4.98	6.86	27.22
Putnam—E	24.79	18.41	1.97	8.09	4.11	5.95	28.47
Susque.—U	21.91	13.26	1.81	10.66	4.48	7.99	25.73
Susque.—O	19.02	13.78	1.76	10.91	4.55	8.12	26.45
Susque.—E	29.22	15.06	1.56	9.84	3.95	7.43	26.46
Cecil—U	31.41	5.03	0.71	14.52	0.59	14.64	20.26
Cecil—O	31.70	5.34	0.76	14.56	0.60	14.72	20.66
Cecil—E	32.58	6.43	0.96	13.09	0.76	13.29	20.48

* X represents the hygroscopic coefficient over 30 per cent H_2SO_4; *Y* represents the hygroscopic coefficient over 3.3 per cent H_2SO_4.

† U, O, and E represent the untreated, oxidized, and extracted colloid, respectively.

The chemical composition of the colloidal material. It is apparent from the foregoing data that the amount of water adsorbed from an atmosphere of water vapor increases with the SiO_2-R_2O_3 ratio of the colloid. The amount of water driven off at high temperatures decreases with this ratio. If one assumes this water to be combined water (a questionable assumption as will be seen later) then it increases with a decreasing SiO_2-R_2O_3 ratio. These relationships which also exist for the experimental data of other investigators (1, 2, 3, 10, 13, 14, 23) are shown in figure 10. As the amount of SiO_2 in the colloidal clay increases, the amount of water which the colloid will adsorb and then release at a temperature of 110°C. becomes larger. As the amount of SiO_2 in the clay decreases, the amount of water which the colloid will release above this temperature increases. The total amount of water which the different colloids contain from that amount they will adsorb over 30 per cent H_2SO_4 to that which they will release at ignition is not so markedly affected by the chemical composition of the colloid, although the Cecil and Susquehanna colloids with low SiO_2 R_2O_3 ratios have a lower total water content. The total water content of the different colloids between these limits may be found in table 6.

These data suggest the question, what justification is there for assuming that the water driven off between 110° and ignition is combined water? It is extremely difficult clearly to define combined water in the light of our present knowledge of the nature of the forces involved in the retention of water by colloidal material. It is obvious, however, that merely setting 110°C. as marking the temperature at which adsorbed water ceases to be given off is unsound. It would seem logical to consider combined water as that water which is a part of the crystal lattice makeup of the colloid. With this consideration the water which is adsorbed as a result of certain attractive forces in the surface of the colloid, irrespective of their nature, would not be combined water. Jenny (16) believes that part of the water of constitution, or combined water, in H-systems is the "potential water molecules" formed by the exchangeable H ions and the OH ions in the alumino-silicate crystal lattice framework.

The H-clay, therefore, should contain the most combined water, a fact which is indicated by the data in this study. Additional experiments with H-systems, which are not reported in this paper, show that the decrease in the number of exchangeable H ions and in the amount of water which a colloid will adsorb as affected by heating are proportional. Although the results of this investigation tend to confirm Jenny's hypothesis, further very carefully controlled experiments must be made before its validity can be definitely established.

If the combined water is a definite part of the crystal lattice makeup then the loss of water from a colloid as affected by heating is not likely to be some simple function of the temperature. One should expect various crystal lattices to give up their water at different temperatures. If the water loss is plotted as a function of temperature there should be a break in the curve. The experimental data in this study as shown in figures 2 and 4 indicate that, with the exception of the permutite, the different colloids lose their water at varying rates dependent upon the temperature. The curves are characterized by minimum and maximum points which may or may not occur at the same temperature, depending upon the colloid. As stated previously, these maximum points are considered to represent an increase in the rate of water loss as a result of the breaking up or shattering of the crystal lattice. If this is true the amount of water removed from a colloid from the minimum point to ignition should be combined water. It is granted, however, that there may be some tightly held adsorbed water which is also given off at these higher temperatures. It is interesting to note, on the other hand, that those colloids with a relatively high surface activity lose much less water at the higher temperatures than those that adsorb comparatively small amounts of water on their surfaces.

Assuming, therefore, that the water which is expelled from the colloid from the minimum point to ignition is the greater part, if not all, of the combined water, one obtains some very interesting relationships with the chemical composition of the colloid. The data in figure 9 show the relation of the oxides of Al, Fe, and Si to the water removed from the different colloids between these limits: (*a*) the amount adsorbed over 30 per cent H_2SO_4 to that driven off at the temperature of the minimum point, and (*b*) the amount driven off between the minimum point and ignition.

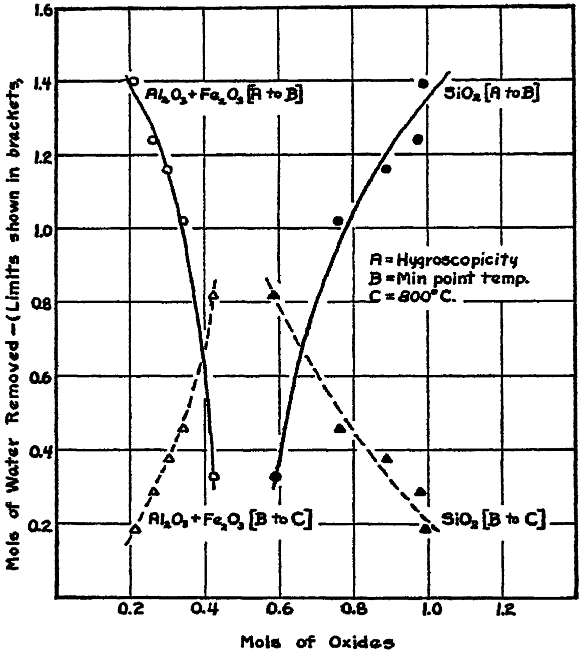

FIG. 9. RELATION OF WATER ADSORPTION TO THE SiO_2, Al_2O_3, AND Fe_2O_3 CONTENT OF SOIL COLLOIDS

If one first considers the solid lines which show the relation between the water loss from hygroscopicity to the minimum point temperature, it is seen that as the mols of $Al_2O_3 + Fe_2O_3$ increase in the colloid the water loss decreases. The opposite is true for the SiO_2 content. If one next considers the dotted lines which show the relation of the water loss from the minimum point temperature to ignition, it is observed that as the mols of $Al_2O_3 + Fe_2O_3$ increase in the colloid the water loss increases. The opposite relationship holds for the SiO_2 content. These data are particularly significant, since the minimum point temperatures of the various colloids are different. (In the case of the Cecil and Susquehanna, the temperature of the first minimum point was chosen.) The data show that the amount of water which is held rather firmly and apparently released rapidly at a certain critical temperature increases with an increase in the Al and Fe in the crystal lattice makeup of the colloidal clay system. An increase in the SiO_2 content of the complex favors the adsorption of water as a result of surface phenomena. Although this concept of combined water may have to be altered to some extent as a result of further experimental investigations it is a definite step to modify our present use of "110° to ignition" as representing this type of water.

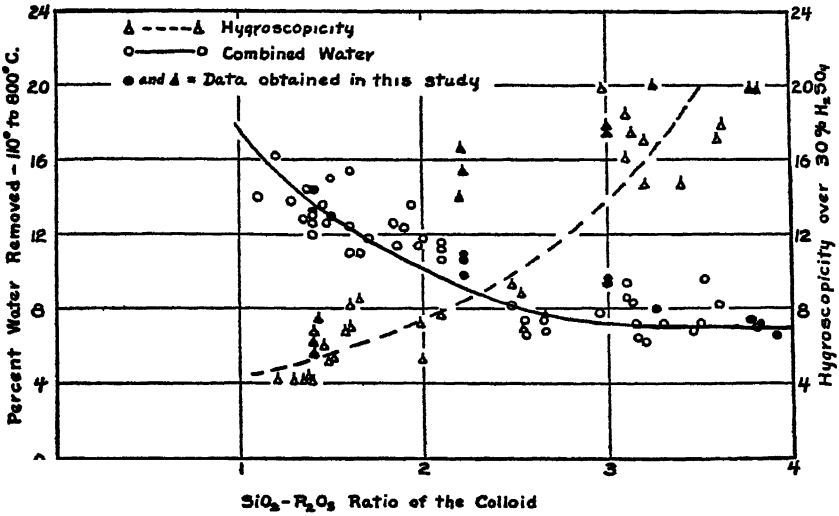

FIG. 10. RELATION BETWEEN THE HYGROSCOPICITY AND COMBINED WATER AND THE SiO_2-SESQUIOXIDE RATIO OF SOIL COLLOIDS

DISCUSSION

The data obtained in this investigation show that the amount and nature of the water in soil colloidal systems are greatly affected by the nature of the colloidal material, its chemical composition, and the exchangeable cations present.

Systems of H-bentonite, H-clay, and H-permutite vary widely in the intensity with which the water is held. The H-bentonite, a fairly close-packed system, loses water the most rapidly at low temperatures; the H-permutite, an open-packed system, loses the most water at the higher temperatures. This may be explained on the basis that the water adsorption in the case of the bentonite is more or less an external surface phenomenon, whereas in the permutite, the adsorption occurs mostly in the large cavities existing throughout the particles. Consequently, the adsorbed water in the permutite is more difficult to remove. The H-clay occupies a position intermediate between the bentonite and permutite.

The curves showing the water loss from these colloids as a function of temperature are sigmoidal and exhibit distinct breaks, with the exception of the permutite. These breaks in the curves are considered as points at which the water held on or in the surface is probably completely removed and at which the crystal lattice of the colloidal particles is beginning to break down. The sudden rise in the rate of water loss curves after the minimum point is pictured as being due to the fracturing of the crystals which causes a more rapid release of the water held in the interior of the particles.

The rate of water loss curves for the H-bentonite and H-Putnam clay exhibit maximum points at about 550° and 400°, respectively. Mixtures of bentonite and Putnam clay exhibit both of these points. These distinctly different maximum points suggest at least two possible explanations. The first is that the alumino-silicates in these two colloidal materials which are responsible for base exchange are distinctly different. The second is that each of these colloidal complexes contain an impurity which contains water of crystallization and is responsible for the maximum points. In order to determine which of these two explanations might be sufficient to account for the experimental facts, samples of these two colloids were heated at the various temperatures until water ceased to be removed. The exchange capacities and the hygroscopicities of the heated samples were then determined. It was found that there was a large decrease in the exchange capacity and water adsorption of both colloids after they had been heated at temperatures higher than that of the minimum point. The constituent of the clay, therefore, which is responsible for the maximum point undoubtedly has base exchange properties.

It was also observed that the percentage decrease in the total exchange capacity of the bentonite per unit increase in temperature was several times that of the Putnam clay. These results indicate very strongly that the complexes in these two colloids which contribute to base exchange are distinctly different and that the maximum points in the rate of water loss curves are caused by constituents of the colloid which possess distinct base exchange properties. The data corroborate other experimental results which indicate that the two colloids may be different. The interesting observation was made that water adsorption was almost reversible as long as the colloid was heated at temperatures below the minimum point.

The rate of water loss curves for the Cecil and Susquehanna colloids are characterized by a minimum point at about 125° and 200°C., respectively. The Susquehanna curve also exhibits a minimum point at about 425°. This indicates that the two colloids contain at least two different types of colloidal material. Hendricks and Fry (12), in studying the X-ray diffraction patterns of the Cecil colloid, state that the halloysite pattern is the most predominant. Halloysitic material would be expected to contain a different amount of water from that of the montmorillonitic constituents which are considered as being present in the Putnam colloid. All of the foregoing data indicate very strongly that the soil colloidal material consists of different types of mineral complexes. Further experiments are being made in the hope of throwing more light on the nature of the base exchange complex in soil colloids.

SUMMARY

Various soil colloids and colloidal alumino-silicates were studied to determine the relationships between their water content and the chemical composition of the colloids, the amount and nature of exchangeable cations present, and the presence of organic matter and free oxides of Al, Fe, and Si.

Organic matter was removed by oxidation with H_2O_2. The free oxides were removed with alternate extraction with 0.1 *N* HCl and 2 per cent Na_2CO_3.

The amount of water adsorbed by the different colloids over 3.3 per cent and 30 per cent H_2SO_4 was determined.

The water loss from the colloids as a function of temperature was studied.

Hygroscopicity over 3.3 per cent H_2SO_4 was not appreciably affected by the nature of the colloid but appeared to be some function of the specific surface.

The hygroscopicity over 30 per cent H_2SO_4 was affected not only by the chemical composition of the colloid but also by the nature of the exchangeable cations on the exchange complex. The amount of adsorbed water increased with the SiO_2-R_2O_3 ratio of the colloid. Clays saturated with various cations increased in their hygroscopicity values according to the series:

$$\mathrm{H} > \mathrm{Ca} > \mathrm{Li} \overset{=}{>} \mathrm{Mg} > \mathrm{Na} \overset{=}{>} \mathrm{Ba} > \mathrm{K}$$

Removal of the small amount of organic matter present in these colloids had little effect on their water content.

The extraction of soluble Al_2O_3, Fe_2O_3, and SiO_2 did not materially change the water content of the colloids, although there was a tendency for the combined water to decrease with free oxide removal.

The temperature-water loss curves, with the exception of permutite, were sigmoidal in shape, with distinct points of inflection. The temperature-rate of water loss curves exhibited maximum and minimum points at certain temperatures. The temperatures at which these points occur apparently depend upon the chemical nature of the colloid. These breaks are interpreted as representing the temperature at which the crystal lattice of some constituent of the colloidal complex is shattered, causing a more rapid loss of water.

The temperature-water loss curves suggest that there are at least two kinds of water retained by the colloid. The arbitrary use of 110°C. as representing the temperature at which all adsorbed water is driven off is seriously questioned.

The suggestion is made that combined water should represent that water which is a part of the crystal lattice of the colloidal complex.

The temperature-water loss curves indicate that the colloidal material extracted from various soils is different. Considerable differences were shown by the bentonite, clay, and permutite curves.

The nature of the exchangeable cations affects the amount of water removed from a Putnam clay at temperatures lower than about 250°C., but have only a slight effect at higher temperatures.

The total water loss, between saturation over 30 per cent H_2SO_4 and ignition, by clays containing different exchangeable cations followed the order:

$$H > Mg = Ca > Li > Ba \gtreqless Na > K$$

Hygroscopicity of the different colloids increased with the SiO_2-R_2O_3 ratio. The combined water decreased with an increasing ratio.

Preliminary observations on the effect of heating bentonite and Putnam clay on their cation exchange capacities suggest that the water loss-temperature curves give some index of the nature of the base exchange complex.

The theory that the same base exchange material is responsible for cation exchange in bentonite and clay is seriously questioned in the light of these investigations.

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A NEW METHOD FOR ESTIMATING REPLACEABLE Na AND K, IN SOILS

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The profound influence of exchangeable ions in modifying the physicochemical properties of soils is well known. A recognition of this fact has greatly helped in clarifying our views regarding alkali soils, the origin of which has been definitely traced to the accumulation of exchangeable Na in them.

Methods of estimating exchangeable ions are too well known to need detailed recapitulation. They can be divided into three classes:—

(*a*) Replacement with neutral salts such as sodium chloride, ammonium acetate, or barium chloride.
(*b*) Replacement with dilute acids such as $0.05N$ HCl, or acetic acid.
(*c*) Electrodialysis.

The actual estimation of individual bases is done, of course, by well-known analytical methods in every case.

The estimation of exchangeable sodium had always presented peculiar difficulties on account of the absence of a satisfactory method for the estimation of this element; the usual procedure being to eliminate all other ions and to determine sodium by difference or evaporation and weighing of the residue. The newer methods of Barber and Kolthoff (1) or Kahn (2) have made possible direct estimation of sodium, but the difficulties of the method are greatly enhanced in soil work by the inevitable presence of large amounts of extraneous ions. Another difficulty is that soils containing exchangeable sodium are generally rich in soluble sodium salts and unless these are washed first (and this must result in hydrolysis of the exchange complex and some replacement of exchangeable sodium by soluble calcium salts), the results are bound to be too high.

A knowledge of the amount of exchangeable sodium is indispensable for the proper understanding of the genesis of alkali soils and their amelioration. In the course of an investigation on the antagonism between Ca and Na ions in soils, a method for the estimation of exchangeable Na and K was worked out which appears of sufficient interest to warrant a separate publication, embodying the main outlines of the method.

The principle of the method is the same as replacement with neutral salts, only in this case the replacing agent is $Ba(OH)_2$ which causes the replacement of Na and K by Ba, the former coming out as hydroxides. Through the filtrate containing the hydroxides of Ca, Ba, Na, and K, carbon dioxide is passed. The heavy metals are thus precipitated as carbonates, and alkali metals are converted into bicarbonates. The solution is boiled and filtered. The filtrate is titrated with standard acid, methyl red being used as indicator.

EXPERIMENTAL

The series of experiments the results of which led to the formulation of this method will be described first in their logical sequence.

In order to see what concentration of $Ba(OH)_2$ solution would be necessary to bring about the replacement and how many washings would be adequate, the following experiment was performed:

A heavy black cotton soil containing 56 per cent clay and 5 per cent $CaCO_3$ was leached with 1,000 cc. of increasingly concentrated NaCl in 100-cc. lots. The leachate was made to volume and Ca determined in an aliquot. It was assumed that, after the solubility effect had been allowed for, an approximately equivalent amount of Na would have gone into the soil. (The soil contained very little exchangeable Mg.) The treated soils were washed with increasing concentrations of $Ba(OH)_2$ in 100-cc. lots, $NaHCO_3$ being determined in each lot as usual. The results given in table 1 show that the whole of the exchangeable Na can be completely replaced by 500 to 600 cc. of 0.2 *N* $Ba(OH)_2$ solution.

TABLE 1

Replacement of Na from soil by $Ba(OH)_2$ solutions

CONCENTRATION OF NaCl SOLUTION USED FOR FIRST LEACHING	Ca IN THE LEACHATE AFTER CORRECTING FOR DISSOLVED AMOUNT	STRENGTH OF $Ba(OH)_2$ SOLUTION	Na IN SUCCESSIVE LEACHINGS OF 100 CC.					
			1	2	3	4	5	Total*
per cent	*m.e.*		*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
2	4.95	0.05*N*	2.66	1.36	0.46	0.27	0.19	3.94
3	4.80	0.10*N*	3.36	1.36	0.51	0.25	0.23	5.66
4	5.30	0.20*N*	4.93	1.11	0.27	0.09	0.09	6.59
5	5.05	0.40*N*	5.34	0.83	0.14	0.03	0.005	6.345

* Since the soil already contained 0.90 m.e. of Na and K, this amount must be subtracted from the total Na given.

In order to determine whether the whole of the exchangeable Na could be replaced by a single treatment of 1,000 cc. of 0.2 *N* $Ba(OH)_2$ solution, another series of NaCl-treated soils was prepared by using smaller concentrations of the salt. The soils after being dried were suspended in 1,000 cc. of 0.2 *N* $Ba(OH)_2$ and left for 2 to 3 hours with occasional shaking. The suspension was allowed to settle, and 100 cc. of the supernatant liquid was pipetted off and treated with CO_2 for NaOH estimation. The results given in table 2 bring out the interesting fact that a single treatment of 0.2 *N* $Ba(OH)_2$ solution may be sufficient to effect the complete replacement of exchangeable Na.

It might be pointed out that for this work soils having very high clay content and exchange capacity were deliberately chosen and saturated with Na to apply the most rigid test to the method. In nature such high values for exchangeable Na are never met with. This is definitely shown by the investigation now proceeding in this laboratory, the results of which will be made available at a later date.

Detailed description of the proposed method

Ten to twenty grams of soil is left with 100 cc. of 0.2 *N* $Ba(OH)_2$ solution for 2 hours with occasional shaking. It is then filtered through filter paper that has been stuck on a buchner funnel by running molten wax round the edges. The soil is then leached with 0.1 *N* baryta in 100-cc. lots, until the filtrate measures 1,000 cc. One or two drops of phenolphthalein indicator is added to the filtrate and CO_2 gas passed through the entire 1,000 cc. till the solution just becomes colorless. The carbonates of Ca and Ba are allowed to settle, which they do in about half an hour. Five hundred cubic centimeters of the supernatant liquid is boiled down to about 50 cc. Carbon dioxide is again passed until the phenolphthalein color is just discharged. Then 25 cc. of approximately 0.05 *N* ammonia solution is added, and the liquid is brought to the boiling point and filtered. The precipitate on the filter paper is washed three or four times with approximately 0.05 *N* ammonia solution. The filtrate is boiled briskly for about half an hour to drive away all the ammonia and titrated, after cooling, against standard acid using methyl orange as indicator. A more satisfactory method of titration is to add excess of standard acid, boil the solution to expel CO_2 and back titrate with standard alkali using ***brom thymol blue*** as indicator.

TABLE 2

Replacement of Na by a single treatment of 1,000 cc. of 0.2N $Ba(OH)_2$ solution

CONCENTRATION OF NaCl SOLUTION USED FOR LEACHING	Ca IN THE LEACHATE (X)	REPLACED Ca ($X - 0.588$)	Na REPLACED BY 0.2*N* $Ba(OH)_2$ (Y)	Na AFTER ALLOWING FOR THE QUANTITY ALREADY PRESENT IN SOIL ($Y - 0.900$)
per cent	*m.e.*	*m.e.*	*m.e.*	*m.e.*
0.00	0.588	0.000	0.900	0.00
0.05	1.274	0.686	1.600	0.70
0.10	1.666	1.078	1.700	0.80
0.15	2.058	1.470	2.200	1.30
0.20	2.450	1.862	2.100	1.20
0.25	2.647	2.059	2.700	1.80
0.30	2.941	2.353	3.000	2.10
0.35	3.333	2.745	3.600	2.50
0.50	3.627	3.039	4.150	3.25
0.75	4.510	3.922	4.900	4.00
1.00	5.098	4.510	5.200	4.30
1.50	5.686	5.098	5.550	4.65

Alternately 10 gm. of soil is suspended in 1,000 cc. of 0.2 *N* $Ba(OH)_2$ solution and left for 2 hours with occasional shaking. The suspension is allowed to settle; 750 cc. of the clear supernatant liquid is treated with CO_2 and allowed to settle; and 500 cc. of the supernatant liquid is boiled down and filtered after the addition of ammonia. The filtrate after being boiled is titrated as described in the foregoing.

The technique described here gave very satisfactory results in control experiments with known amounts of NaOH in the presence of Ca and Ba hydroxide. A typical set of such determinations is given in table 3.

Chlorides to the extent of 10 per cent in the soil have no effect on the determination; but if sulfates are present they should be determined separately by shaking the soil with NaCl solution, allowing it to settle and estimating the sulfates in an aliquot of the supernatant liquid. An amount of $BaCl_2$ equivalent to the sulfate determined is then added to the soil. The Na_2SO_4 is thus converted into NaCl and the soil is treated with 0.2 *N* $Ba(OH)_2$ as usual. Since Na_2SO_4 is quantitatively converted into insoluble $BaSO_4$ and soluble NaOH on the addition of $Ba(OH)_2$, an amount equivalent to the sulfate present can be subtracted from the exchangeable Na obtained. If the soil contains appreciable amounts of $CaSO_4$, however, the addition of $BaCl_2$ is the safest course.

TABLE 3

Accuracy of the method of estimating NaOH in the presence of $Ca(OH)_2$ *and* $Ba(OH)_2$

NUMBER	NaOH	
	Present	Found
	m.e.	*m.e.*
1	0	0.025, 0.01
2	0.2	0.211
3	0.3	0.293, 0.306
4	0.4	0.426
5	0.5	0.502
6	1.0	1.003
7	1.5	1.504
8	2.0	2.003
9	3.0	2.997
10	4.0	3.995
11	6.0	5.980
12	8.0	7.996

It might be pointed out that the method gives total monovalent bases, i.e., both K and Na. These can be determined individually as follows:

To an aliquot of the filtrate, after the CO_2 treatment, add ammonia in slight excess, then ammonium carbonate, and finally a small quantity of ammonium oxalate. Filter and wash the precipitate if formed. Transfer the filtrate and washings to a platinum or porcelain dish, add some sulfuric acid, and evaporate to a convenient volume. Then transfer to a platinum crucible and evaporate to dryness. Ignite and weigh the residual K and Na sulfate. The weights of sodium and potassium present in the residue may then be calculated as follows:—

If X = the weight of Na_2SO_4 in the mixed sulfate,
Y = the weight of K_2SO_4 in the mixed sulfate
a = the total weight of the mixed sulfate,
b = the total weight of SO_4 in the mixed sulfate (equivalent to the total carbonates determined by titration)

then:

A)
$$X + Y = a;$$
and
$$\frac{SO_4}{Na_2SO_4} X + \frac{SO_4}{K_2SO_4} Y = b,$$
or,
$$\frac{96.06}{142.06} X + \frac{96.06}{174.26} Y = b$$

(B)
$$0.6762\,X + 0\ 5512\,Y = b$$
Multiplying equation (A) by 0.6762:

(C)
$$0.6762\,X + 0.6762\,Y = 0\ 6762a$$
subtracting (B) from (C):
$$0.125\,Y = 0\ 6762a - b$$
or
$$Y = \frac{0\ 6762a - b}{0\ 125}$$
and from equation (A):
$$X = a - Y = \frac{b - 0.5512a}{0.125}$$
From these values of X and Y the weights of Na_2SO_4 and K_2SO_4 in the mixed sulfates may be found.

If some chlorides are present, they are determined separately, and an equivalent weight of Na_2SO_4 is subtracted from the weight of the residue. On the whole it is just as convenient and indeed preferable to determine K directly in the filtrate, by any of the well-known methods.

SUMMARY

A method for the estimation of exchangeable K and Na in soils has been described. It makes use of $Ba(OH)_2$ as the replacing agent; K and Na appear in the leachate as hydroxides and are determined by titration after Ca and Ba carbonates have been precipitated by passing CO_2.

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THE EFFECT OF ADDITIONS OF CERTAIN COLORADO SOILS UPON THE NITROGEN BALANCE OF A TENNESSEE SOIL

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A number of publications by Headden and by Sackett have shown that nitrates accumulate in detrimental quantities in certain Colorado soils (2, 3, 4, 5, 6, 7, 8). In some instances this accumulation is accompanied by abnormal quantities of chlorides and sulfates. This unusual condition was attributed to the "combined action of nitrogen-fixing, ammonifying, and nitrifying organisms," but this explanation has not been accepted universally. The present contribution, however, does not deal with the well-known controversy as to the causes for the definitely established effect. The objective was to determine whether, with substantial additions of the Colorado soils, nitrogen accumulation and related properties of these soils would be imparted to Tennessee soils and maintained therein under humid conditions. The outline of the project was submitted to Doctor Sackett, who generously supplied the two Colorado soils of known history, one of which had attained toxic concentration of soluble salts.

EXPERIMENTAL

On the basis of mechanical analysis by the Bouyoucos hydrometer method (1), the Tennessee, Grand Junction, and Rocky Ford soils were classified as a clay, a sandy loam, and a clay, with pH values of 6.7, 7.3, and 7.5, respectively. The total nitrogen contents of the Tennessee, Grand Junction, and Rocky Ford soils were 0.122, 0.145, and 0.140 per cent, respectively. The total water-soluble nitrogen was found to be 21, 485, and 90 p.p.m. of moisture-free soil, for the Tennessee, Grand Junction, and Rocky Ford soils, respectively. No difference was found between the amount of nitrogen extracted from each moist soil and that extracted from the quickly dried soil.

The Tennessee soil was a carbonate-free, slightly acid residual soil from dolomitic limestone and had a low content of bases. Both Colorado soils were strongly calcareous; comparable in their total contents of calcium and magnesium, but not in the amounts soluble in either water or dilute HCl. The Grand Junction soil was heavily impregnated with nitrates, chlorides, and sulfates and evidently contained considerable quantities of either undisintegrated dolomite or high-magnesic limestone, whereas in the Rocky Ford soil a large part of the acid-soluble magnesium was in the form of silicates.

Each experimental mixture was made by using 100 pounds, moisture-free basis, of a Cumberland clay loam and 1 pound of a Colorado soil. Full charges, 100 pounds moisture-free basis, of each of the two Colorado soils were also run in parallel. The mixtures and duplicate controls of the untreated Tennessee soil, all without subsoil, were placed in lysimeters of 1/20,000-acre area. These were compared with similar mixtures that received supplementary treatments, (*a*) limestone, (*b*) red clover hay, (*c*) limestone and red clover hay, and (*d*) limestone, red clover hay, and sodium chloride. The heavy limestone addition was intended to assure for the Tennessee soil a reaction comparable to the reactions of the Colorado soils. The large incorporation of clover hay was made to provide a source of energy and an ample supply of oxidizable nitrogenous matter. Since one of the Colorado soils was naturally impregnated heavily with chlorides, the limestone-clover-hay series was paralleled with a series to which was added a 500-pound supplement of sodium chloride.

It was deemed advisable to afford a preliminary contact between the Tennessee soil and the added soils. Accordingly, all of the combinations, with their supplemental additions incorporated throughout, were placed in the outdoor lysimeters November 22, 1927, and protected against rainfall until February 1, 1928. At the end of this 70-day "inoculation period," or "acclimitization" without leaching, the soils were exposed to rainfall leaching for a period of 4 years. The rainfalls for the 4 years were 58.97, 51.06, 41.92, and 44.50, or an average of 49.11 inches.

The nitrogen leached as ammonia, as nitrites, and as nitrates, was determined periodically. Instability of the nitrogen components in the accumulating percolates was minimized by toluene additions. The terminals of the block-tin drainage tubes were "goose-necked" into water traps, so that air would not diffuse up into the soil.

OUTGO OF AMMONIA, NITRITES, AND NITRATES

The outgo of nitrates was determined by the analysis of 31 collections of leachings, 10 during the first year, nine during the second, and 6 during each of the third and fourth years. Ammoniacal and nitrite determinations upon the teachings were run in parallel with nitrates during the first year, after which the nitrogen content of all of the leachings was found to be exclusively in the nitrate form. The totals for each annual period are given in table 1.

The amounts of both ammonia and nitrites leached from the full charges of the Colorado soils were greatly in excess of those found in the leachings from the untreated Tennessee soil. The occurrences of ammoniacal nitrogen in the leachings from the mixtures of the unsupplemented group and those of the limestone group were small, although somewhat greater than the quantity obtained from the untreated Tennessee soil. The largest quantities of ammonia came from the three groups that received clover hay, and no consistent effect was produced by either the limestone or the chloride supplements. The amounts of nitrites were uniformly small, but the meager quantities found for the first group were increased by all supplementary treatments. The pH

TABLE 1

Outgo of nitrogen as ammonia, nitrites, and nitrates from a Tennessee Cumberland loam with a constant inoculation of 1 per cent of Colorado soils, with and without supplements of limestone, red clover hay,† and sodium chloride‡ during a 4-year period*

SOILS AND TREATMENTS	NITROGEN, PER 2,000,000 POUNDS OF SOIL, MOISTURE-FREE BASIS							EFFECT OF COLORADO SOILS UPON OUTGO OF TOTAL NITROGEN
	Ammonia N	Nitrite N	Nitrate nitrogen					
	1928–29	1928–29	1928–29	1929–30	1930–31	1931–32	1928–32	1928–32
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	
Tenn. alone	0.66	0.05	138	63	66	48	315	....
Tenn. + Grand Junction	1.81	0.12	148	68	60	45	321	+7.2
Tenn. + Rocky Ford soil	0.59	0.05	156	56	63	39	314	−1.1
Tenn. + limestone	2.00	0.57	225	77	62	58	422	....
Tenn. + limestone + Grand Junction	2.10	0.13	231	84	72	74	461	+38.7
Tenn. + limestone + Rocky Ford	1.08	0.13	199	90	66	64	419	−4.4
Tenn. + clover hay	5.44	0.45	242	88	85	59	474	....
Tenn. + clover hay + Grand Junction	12.10	0.84	244	89	98	67	498	+31.1
Tenn. + clover hay + Rocky Ford	7.38	0.47	239	85	84	60	468	−4.0
Tenn. + clover hay + limestone	8.52	1.07	392	94	71	73	630	....
Tenn. + clover hay + limestone + Grand Junction	10.86	1.42	351	84	57	63	555	−72.3
Tenn. + clover hay + limestone + Rocky Ford	11.30	0.50	389	102	61	83	635	+7.2
Tenn. + hay + limestone + sodium chloride	10.26	0.61	356	80	74	68	578	....
Tenn. + hay + limestone + NaCl + Grand Junction	11.49	0.65	403	82	68	74	627	+50.3
Tenn. + hay + limestone + NaCl + Rocky Ford	7.60	0.60	373	102	82	59	616	+35.3
Grand Junction soil alone	20.68	9.43	1,188	65	38	33	1,324	....
Rocky Ford soil alone	4.53	0.25	268	87	33	39	427	....

* Three-ton CaO ≎.

† Twelve tons, moisture-free basis.

‡ Five hundred pounds.

values of the leachings from all of the mixtures were almost invariably in excess of 7.

An abnormal nitrifying efficiency was indicated for the Grand Junction soil because of the total outgo of 1,324 pounds per acre of nitrate nitrogen from the full charge of that soil. This represents almost 50 per cent of the initial nitrogen content. But the first collection of leachings between February 1 and April 7, 1928, removed 1,083 pounds of nitrate nitrogen, or 81.8 per cent of the aggregate amount leached during the 4-year period. During the first year 89.7 per cent of the total nitrate outgo was obtained, whereas the losses for the third and fourth years were uniformly less than those from the uninoculated Tennessee soil. Although nitrification in the Grand Junction soil, as measured by outgo of nitrates, was no greater than that of the uninoculated and unsupplemented Tennessee soil after the first year, the residual amount of nitrogen in the Grand Junction soil was only about one-half of that present in the Tennessee soil. It is evident, however, that in its new environment the Grand Junction soil did not accumulate any determinable amount of nitrate nitrogen.

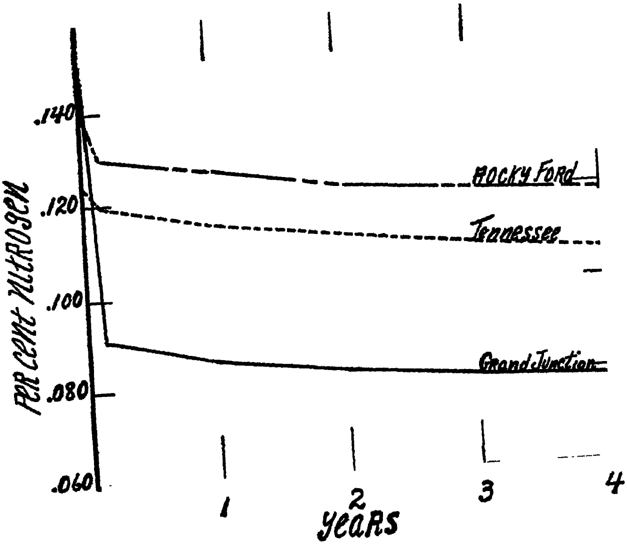

Fig. 1. Progressive Decrease in Nitrogen Content of Untreated Tennessee Cumberland Clay Loam, Grand Junction, and Rocky Ford Soils, as Shown by Nitrate Outgo

The total loss of nitrates from the full charge of the Rocky Ford soil, without treatment, was only 112 pounds in excess of that from the untreated Tennessee soil, and nearly all of this difference occurred during the first year. Of the total outgo of 427 pounds of nitrate nitrogen for the 4-year period, 197 pounds, or 46 per cent, was removed in the first collection of leachings.

The progressive decrease in nitrogen content, as measured by the outgo of nitrates from the controls of the three soils, is shown in figure 1. Subsequent to the early removal of nitrates the configurations are almost identical for the Tennessee and Colorado soils.

The nitrate outgo from the first group, without supplements, was apparently unaffected by the added Colorado soils. The Grand Junction soil gave an enhanced outgo in each group except the fourth, to which was added clover, hay, and limestone. The enhancements were somewhat in excess of the amount of nitrates incorporated. Only in the fifth group was there found an appreciable increase in outgo for the addition of the Rocky Ford soil.

The 12-ton addition of red clover hay (moisture free basis) contained 638 pounds of nitrogen. From this increment increases of only 55, 38, and 56 pounds in total nitrogen outgo were obtained as the effect of limestone supplements to the clover hay in the uninoculated soil, Grand Junction, and Rocky Ford additions, respectively. The highest recovery of total nitrogen in the leachings was 646.8 pounds for the Rocky Ford inoculations of the limestone-clover-hay treatments, or 226.6 pounds more than was obtained from the same combination without clover hay. This is equivalent to 35.5 per cent of the nitrogen content of the clover addition. But the difference between the total nitrogen outgo from the uninoculated Tennessee soil with the two treatments, clover hay alone and clover hay plus limestone, was 159.7 pounds, or 25 per cent of the added nitrogen. The direct effect of the limestone in causing an increase in nitrogen outgo was, however, greater than the inoculation effect. The Grand Junction addition carried 1,546 pounds of carbonates, as $CaCO_3$, per 2,000,000 pounds of soil, and the Rocky Ford addition carried 664 pounds. Since limestone produced a marked increase in nitrogen outgo from the native supply of soil nitrogen, primarily during the first year, and also the same result during the first 2 years for the nitrogen added as clover hay, a part or even all of the effect produced by the Grand Junction soil on the clover hay may have been due to its carbonate content.

The last column in table 1 shows the variations in *total nitrogen* outgo. Small variations were found for the Rocky Ford inoculation within each of the series save the fifth. On the other hand, the Grand Junction comparisons show one small variation, three appreciable plus values and one considerable minus value, the latter being the largest difference obtained. This minus value of 72.3 pounds for the Grand Junction inoculation in the clover hay-limestone group was cumulative, the outgo running consistently low from the beginning of the 4-year leaching period.

When the leachings of total nitrogen for the 4-year period are averaged, the five pairs of uninoculated Tennessee units show a loss of 489.7 pounds as against 500.7 pounds for the five inoculations with the Grand Junction soil and 496.3 pounds for the five Rocky Ford units. Similar averages for outgo of nitrate nitrogen give 484, 492, and 492 pounds respectively. Since the increases shown for the addition of the two Colorado soils are practically identical with the respective amounts of leachable nitrogen introduced by the 1 per cent additions, it is evident that no increase in bacterial activity is recorded for the admixtures.

TOTAL SOIL NITROGEN DETERMINATIONS

At the end of the 4-year period of leaching, the total nitrogen contents of the stored original samples were determined by the modified Gunning method and compared with the residual nitrogen contents of the leached lysimeter soils (table 2). When leached with water in the laboratory at the close of the experiment, the total nitrogen content of the stored Grand Junction soil was reduced from 0.145 per cent to 0.084 per cent, or 42 per cent. The water-soluble fraction of the Rocky Ford soil was 0.011 per cent, or 7.9 per cent of the 0.140 per cent total. The aqueous extracts of both *stored* soils were devoid

TABLE 2

*Total nitrogen content of Tennessee-Colorado soil combinations before and after 4 years leaching by rainfall**

SOILS AND TREATMENTS	TOTAL NITROGEN CONTENT					
	Initial†	After 4 years outdoor lysimeter leaching				
		Found	Loss		Loss induced by Colorado inoculant	
		per cent	*per cent*	*lbs.*	*per cent*	*lbs.*
Tenn. soil alone	0.122	0.105	0.017	340	.....	...
Tenn. + Grand Junction	0.123	0.103	0.020	404	0.003	64
Tenn. + Rocky Ford	0.123	0.106	0.017	343	000	0
Tenn. + limestone	0.122	0.097	0.025	500	.....	...
Tenn. + limestone + Grand Junction	0.123	0.095	0.028	566	0.003	66
Tenn. + limestone + Rocky Ford	0.123	0.095	0.028	566	0.003	66
Tenn. + clover hay	0.154	0.115	0.039	780	.....	...
Tenn. + clover hay + Grand Junction	0.155	0.113	0.042	848	0.003	68
Tenn. + clover hay + Rocky Ford	0.155	0.114	0.041	828	0.002	48
Tenn. + clover hay + limestone	0.154	0.108	0.046	920	.....	...
Tenn. + clover hay + limestone + Grand Junction	0.155	0.105	0.050	1,010	0.004	90
Tenn. + clover hay + limestone + Rocky Ford	0.155	0.106	0.049	990	0.003	70
Tenn. + hay + limestone + sodium chloride	0.154	0.106	0.048	960	.....	...
Tenn. + hay + limestone + NaCl + Grand Junction	0.155	0.101	0.054	1,091	0.006	131
Tenn. + hay + limestone + NaCl + Rocky Ford	0.155	0.104	0.051	1,030	0.003	70
Grand Junction soil alone	0.145	0.075	0.070	1,400	.....	...
Rocky Ford soil alone	0.140	0.105	0.035	700	.....	...

* By modified Kjeldahl-Gunning method.

† Computed from analyses of Tennessee soil plus increment from Oregon soils and red clover hay: basis 2,000,000 pounds plus 20,000 pounds inoculant.

of determinable quantities of either NH_4 or NO_2. A 1,400-pound loss of total nitrogen from the full charge of the Grand Junction soil was shown by the digestion analysis of the original and residual soils, as against a 1,354-pound loss by the leachings. In a similar comparison for the full charge of the Rocky Ford soil there is a marked discrepancy between the data obtained by leaching and by Gunning determinations, the former showing 432 pounds and the latter 700 pounds, as an average of eight determinations.

All five of the Grand Junction inoculation mixtures showed a greater loss than was found for the uninoculated Tennessee soil, and the Rocky Ford inoculation mixtures showed the same result for four of the five series, the exception being the Tennessee soil with inoculation and no supplementary treatment. The previously noted exception for the enhancement in leaching outgo induced by the Grand Junction soil in the clover-plus-limestone series does not show in the analysis for the totals of initial and residual nitrogen.

The largest losses are those of the fourth and fifth groups, identical in treatment save for the single sodium chloride addition in the latter group. When from the averages for the corresponding treatments of these two clover-hay-limestone groups are subtracted, the corresponding losses induced by limestone alone in the second group, the losses of nitrogen from the uninoculated soil, Grand Junction inoculation, and Rocky Ford inoculation are 440, 485, and 444 pounds, respectively. Hence, when the combinations were limed, the large addition of organic matter to supply energy for the organisms that might be carried by the inoculations resulted in only a 45-pound increase in the amount of nitrogen lost in the case of the Grand Junction soil and practically none (4 pounds) from the Rocky Ford soil. The average loss of total nitrogen, for the five uninoculated mixtures, was 700 pounds, as against 784 pounds and 751 pounds for the Grand Junction and Rocky Ford inoculations, respectively. The greater differences shown by the Gunning method may be due to analytical error inherent to the procedure, or it may be that the digestion method registers an actual loss through denitrification that would not be registered as such by the leaching data.

SUMMARY AND CONCLUSIONS

It seemed possible that the incorporation of an ample amount of a Colorado soil of high nitrifying and fixation capacities would impart such properties to a Tennessee soil. The outgo of ammoniacal, nitrite, and nitrate nitrogen from the original Colorado soils and their 1 per cent admixtures with a Tennessee Cumberland clay loam were therefore studied by means of lysimeters during a 4-year period. It was thought that this period would be ample to permit the inoculations to become "acclimitized" and effective, if they were to impart greater bacterial activities to the Tennessee soil. To assure the organisms a medium of a reaction similar to that of their native habitat, a 6,000-pound CaO-equivalent of finely-ground limestone was used as one of the treatments, with and without a 12-ton addition of red clover hay to serve as a source of energy and nitrification. Since one of the Colorado soils was well supplied with chlorides, a 500-pound supplement of NaCl was also used in one series. The lysimeter leachings, collected in the presence of toluene, were obtained from an 8-inch depth of soil, without subsoil.

During the first year there was considerable outgo of ammoniacal and nitrite nitrogen from the Grand Junction soil, and the outgo of ammoniacal nitrogen from the Rocky Ford soil was materially greater than that from the Tennessee soil. This was true also from the admixtures, especially where clover hay supplements were used. In all cases the outgo was exclusively in the nitrate form after the first year.

In four of the five group treatments, the outgo of nitrates and of total nitrogen was increased in relatively small amounts by the nitrate-impregnated and strongly alkaline Grand Junction soil, but the Rocky Ford soil failed to cause any such increase in four of the five groups.

When measured by Kjeldahl-Gunning determinations, a greater loss of total nitrogen was indicated for each addition of the Grand Junction soil than that shown by the leaching data, and the same held for four of the Rocky Ford additions. This indicates possible denitrification.

The maximum recovery of the nitrogen added as clover hay was 52 per cent of the addition. Since the limestone supplements were more effective than any inoculation treatment in causing increased outgo of nitrogen from both native and added supplies, the alkalinity induced by the soil additions was probably a factor in producing enhancements in nitrogen outgo.

It is evident that the unusual properties of the Colorado soils were not maintained, nor were they transmitted to the Tennessee soil under any of the experimental conditions imposed.

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A RAPID METHOD FOR IDENTIFYING THE BACTERIA IN NODULES OF LEGUMES

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While conducting some other work on the legume organisms our attention was repeatedly called to the danger of contamination or chance infection in greenhouse experimentation. Some rapid and dependable method is desired by which the identity of the organisms in the nodule may be obtained without the necessity of plating out and isolating the organisms.

HISTORICAL

Zipfel (6) in 1912 first used the agglutination method for studying the nodule bacteria. He carried out a large number of experiments, two of which are pertinant to this investigation. In his studies on the pea bacteria he used immune serum prepared by injecting pure cultures of pea bacteria into rabbits and for antigen he used both nodule contents suspended in physiological salt solution (Agglutinationversuch 5) and pea bacteria in pure cultures likewise suspended in physiological salt solution (Agglutinationversuch 6). The results were the same for both antigens, the nodule contents gave equally as high titre as the pure cultures of the pea bacteria.

Bialosuknia and Klott (1) observed that only one seriological strain of the nodule bacteria was to be found in a single nodule, though on the same plant, nodules could be produced by bacteria of different serological behavior.

For the diagnosis of Bang's abortion disease Huddleson and Abell (3) devised a rapid macroscopic agglutination test in which they used a heated standardized suspension of *Brucella abortus* in 12 per cent sodium chloride solution with different amounts of undiluted serum on a glass cover of an especially lighted box.

In a recent paper by Hansen and Tanner (2) both the slow and the rapid macroscopic agglutination methods were used to identify the organisms obtained from nodules resulting from cross inoculation studies.

The purpose of this investigation was to combine the method of Zipfel with that of Huddleson and Abell in identifying the causal organisms in nodules produced in cross inoculation experiments. This obviates the necessity of plating out the nodules and reduces the time and labor required by the slow macroscopic agglutination method.

METHODS

Immune sera were prepared as described in a previously mentioned paper by Tanner and Hansen.

The antigens were prepared by first sterilizing carefully the outside of the nodule and then crushing one nodule in about 3 cc. of a 2 per cent physiological salt solution, removing the coarse nodule fragments by decantation.

The apparatus consisted of a Wolffhuegel counting plate or similar piece of glass set up so that it could be illuminated and warmed from beneath by means of a miniature microscope light or other light source.

Circles about 1½ inches in diameter were drawn on the glass plate with a wax pencil, and just inside this line forming the circle a band of petrolatum was drawn with a cotton swab, in order to prevent the liquid of the several tests on the plate from running together.

Antigens were placed in the proper circles with 10-cc. pipettes which delivered approximately 15.5 drops per cc.; three drops were placed in each circle.

Immune sera diluted 1:10 with physiological salt solution were added to and mixed with the proper antigen with a standard loop delivering 0.01 cc. This gave a titre of about 1:200, which was found after some preliminary trials to be very satisfactory.

Readings were made in 15 to 30 minutes. Occasional agitation of the plates with a rotary motion seemed to hasten the reaction and to facilitate readings.

TABLE 1

Cross inoculation with navy bean and dalea bacteria

FLASK	SEED	INOCULATION	NODULES
67	Navy bean	60 Navy bean	30 large, a few small
68	Navy bean	70 Dalea	21 large, a few small
69	Navy bean	None	None

EXPERIMENTATION

Experiment 1

The production of nodules on navy bean roots by dalea nodule bacteria as reported by Sears and Clark (5) was first subjected to investigation. Navy bean seeds were planted in 1-liter Erlenmeyer flasks. The technic used in the growth of the plants in these flasks is described in a previous paper. The seeds were planted and inoculated October 18, 1929, and the plants were washed out and examined December 16, 1929. The results are indicated in table 1.

The nodules found in 67 and 68 were then subjected to examination by the rapid agglutination method. Four large nodules were taken from each flask, each nodule being crushed in 2 per cent salt solution. Immune sera were prepared from pure cultures of navy bean and dalea organisms by the previously described method. These results appear in table 2.

Experiment 2

That soybean and cowpea bacteria were interchangeable in nodule forming function was reported by Leonard (4), though previously they had been placed in separate cross inoculation groups by several investigators. This particular cross was examined.

Soybeans and cowpea seeds were planted in 1-liter Erlenmeyer flasks as in the previous experiment. The seeds were planted and inoculated on January 1, 1930, and the plants were washed out and examined on February 12, 1930. The results obtained are shown in table 3.

The nodules from flasks 89, 91, and 92 were examined by the rapid agglutination method. Four large nodules from each flask were again selected and crushed in 2 per cent salt solution. The suspensions were tested for agglutination in immune sera prepared with pure cultures of cowpea and soybean bacteria. The results of this test appear in table 4.

TABLE 2

Agglutination tests with crushed nodules as antigens and immune sera prepared from pure cultures of the dalea and navy bean organisms

SOURCE OF ANTIGEN	IMMUNE SERA	
	Navy Bean 60	Dalea 70
Flask 67, navy bean:		
Nodule 1	+	–
Nodule 2	+	–
Nodule 3	+	–
Nodule 4	+	–
Flask 68, dalea:		
Nodule 1	–	+
Nodule 2	–	+
Nodule 3	–	+
Nodule 4	–	+

+ indicates positive agglutination; – indicates no agglutination.

TABLE 3

Cross inoculation with cowpeas and soybean bacteria

FLASK	SEED	INOCULATION	NODULES
89	Soybean	55 Soybean	7 large, a few small
90	Soybean	34 Cowpea	None
91	Cowpea	55 Soybean	4 large, a few small
92	Cowpea	34 Cowpea	14 large, a few small

Experiment 3

The foregoing technic was then used in an attempt to verify the observations of Bialosuknia and Klott that on the same plant, nodules could be produced by bacteria of different serological properties. A cowpea seed was planted in a 1-liter Erlenmeyer flask and inoculated with a suspension of approximately equal amounts of serologically distinct cowpea organisms (Cowpea 34 and ICT Cowpea). Ten nodules on the cowpea plant were crushed in separate tubes containing 2 per cent salt solution, and the suspensions were

Agglutination test with crushed nodules as antigens and immune sera produced from pure cultures of soybean and cowpea organisms

SOURCE OF ANTIGEN	IMMUNE SERA	
	34 Cowpea	55 Soybean
Flask 89, soybean:		
Nodule 1	−	+
Nodule 2	−	+
Nodule 3	−	+
Nodule 4	−	+
Flask 91, cowpea:		
Nodule 1	−	+
Nodule 2	−	+
Nodule 3	−	+
Nodule 4	−	+
Flask 92, cowpea:		
Nodule 1	+	−
Nodule 2	+	−
Nodule 3	+	−
Nodule 4	+	−

+ indicates positive agglutination; − indicates no agglutination.

TABLE 5

Agglutination tests of the nodules on cowpea plants inoculated with two strains of organisms, and their immune sera

	IMMUNE SERA	
	34 Cowpea	ICT Cowpea
1	+	−
2	+	−
3	+	−
4	+	−
5	+	−
6	+	−
7	+	−
8	+	−
9	+	−
10	+	−

TABLE 6

Agglutination tests of the nodules on soybean plants inoculated with two distinct strains of organisms, and their immune sera

	IMMUNE SERA—55 SOYBEAN
1	+
2	+
3	+
4	+
5	+
6	−
7	+
8	+
9	+
10	+

tested for agglutination in immune sera prepared from the original pure cultures of the cowpea organisms. The results appear in table 5.

Similarly, a soybean seed was inoculated with two serologically distinct strains of soybean bacteria (No. 54 and No. 55). Ten nodules were tested for agglutination, though only one serum was used (No. 55) the supply of the other (No. 54) having been used up in other work. The results are reported in table 6.

It will be observed that the suspension from one of the nodules gave no agglutination with the serum used. The negative result was confirmed by using a serum of a soybean strain obtained from a different source (No. 920) but identical in serological behavior. This would indicate that the nodule was produced by strain No. 54, which would confirm the results of Bialosuknia and Klott, though it is unfortunate that serum No. 54 was not available to verify the result.

DISCUSSION

In the inoculation experiments as reported in table 1 the results confirmed again those reported by Sears and Clark, that the dalea organisms are able to produce nodules on the navy bean plant. Likewise the inoculation experiments reported in Table 3 are consistent with those reported by Leonard in that the soybean nodule bacteria readily produce nodules on the cowpea plant, the reciprocal cross being not so readily demonstrated.

In the agglutination tests as recorded in tables 2 and 4, the rapid method herein described gave quick and dependable verification of the cross inoculation results by identifying the organisms in the nodules.

That nodules on the same plant may be produced by bacteria of different serological behavior as reported by Bialosuknia and Klott could not be completely verified. In the first test (*see* table 5) all 10 nodules contained the same serological strain. In the second test, nine nodules contained the same serological strain, the tenth being dissimilar. Complete identification of the strain could not be obtained in the latter case, as the necessary serum was not available. The results of these experiments do show, however, that the use of nodule suspension in the rapid agglutination method does give a quick and dependable means of identifying the causal organism.

CONCLUSIONS

A rapid and dependable method for identifying the organisms in the nodules of legumes is obtained by using the nodule contents as antigen, as described by Zipfel, in agglutination tests after the method of Huddleson and Abell. This method is of particular value in verifying the results in cross inoculation experiments where strains of different serological behavior are used.

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FURTHER STUDIES ON THE RELATION BETWEEN THE CARBON ASSIMILATION AND NITROGEN FIXATION IN LEGUMINOUS PLANTS[1]

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In a previous paper (9) it was reported that the addition of CO_2 to clover and alfalfa plants resulted in an increase in the quantity of nitrogen fixed. Since this report, additional experiments have been completed. These studies relate to the following two aspects of the question: (*a*) the effect of pCO_2 compared with total quantity of CO_2 available to the plant; (*b*) the effect of added carbohydrate on nitrogen fixation by nodulated leguminous plants.

PART I. COMPARISON BETWEEN pCO_2 AND TOTAL QUANTITY

Methods

The methods employed for growing the plants for and analyses were described in earlier publications (1, 10).

Experimental

In the experiments discussed in the first paper (9), it was observed that even a small increase in the pCO_2 produced large increases in the dry weight, nitrogen fixed, and nodules formed by clover plants. Calculations of the total quantity of carbon dioxide supplied the plants during the period of growth showed that in the series receiving air, about 40 per cent of the total carbon furnished the plant as CO_2 was recovered in the plants.[2] In the 0.1 per cent CO_2 series, only 25 per cent of the total carbon available was recovered in the plants, whereas in the 0.2 to 0.8 per cent series the recovery was much less, ranging from 5 to 15 per cent. It was thought possible that the marked initial benefit to clover of increased CO_2 supply was due to increase in *total quantity* of CO_2 rather than pCO_2. In order to settle this question, two experiments were made in which the total quantity of CO_2 available to the plants was kept constant but the pCO_2 was changed.

Experiment I.—In this experiment air (0.03 per cent CO_2) was supplied to one series of clover plants grown in 64-ounce bottles (sand or agar) at the rate of 125 cc. per minute per bottle. In a second series, the pCO_2 was maintained at 0.1 per cent, but the rate of aeration was lowered to 37.5 cc. per minute per bottle. Thus each bottle of 10 clover plants received about 2.25 cc. CO_2 per hour but the pCO_2 was varied 3.3 fold. After 65 days, analyses were made; these are given in table 1, experiment I.

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[1] Herman Frasch Foundation in Agricultural Chemistry, Paper No. 57. Contribution from the Departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin.

[2] Assuming plants are 50 per cent C on dry basis.

Experiment II.—A second experiment was made in which the total quantity of CO_2 received per bottle per hour was increased to approximately 3.4 cc. This was done by increasing the rate of aeration to 55 cc. per minute per bottle in the 0.1 per cent CO_2 series and to 187 cc. per minute per bottle in the air series. In this experiment, frequent analyses were made on the CO_2 content of the atmospheres by the potentiometric method described elsewhere (12), in order to insure that the CO_2 content in the series did not vary markedly from the desired values. After 49 days, the analyses given in table 1, experiment II, were made. In this experiment, a mold infection invaded the air series on agar and destroyed the plants. The 0.1 percent CO_2 series was kept free of the infection, and the results of the analyses are included for comparison with the sand series

TABLE 1

Comparison of effect of total CO_2 with pCO_2 on growth and fixation of elemental nitrogen by clover

TREATMENT	RATE OF AERATION*	NUMBER OF SAMPLES	NUMBER OF NODULES	DRY WEIGHT	NITROGEN	
					Total	Per cent
	cc./min.			mgm.	mgm.	
Experiment I, 3–15–32 to 5–19–32						
Sand series:						
0.1 per cent CO_2	37.5	8	133 ± 9†	798 ± 46	21.6 ± 1.3	2.77 ± 0.08
Air (0.03 per cent CO_2)	125.0	8	99 ± 7	308 ± 18	9.2 ± 0.6	2.97 ± 0.10
Agar series:						
0.1 per cent CO_2	37.5	7	550 ± 69	776 ± 55	23.8 ± 1.8	3.07 ± 0.07
Air (0.03 per cent CO_2)	125.0	8	246 ± 32	351 ± 21	11.1 ± 0.6	3.16 ± 0.01
Experiment II, 6–23–32 to 8–11–32						
Sand:						
0.1 per cent CO_2	56.0	8	75 ± 5	544 ± 18	12.4 ± 0.5	2.28 ± 0.06
Air (0.03 per cent CO_2)	187.0	6	57 ± 6	357 ± 38	8.1 ± 1.0	2.26 ± 0.06
Agar**:						
0.1 per cent CO_2	56.0	5	157 ± 42	476 ± 21	13.1 ± 0.7	2.77 ± 0.15

* Per bottle.

** Air series attacked by mold infection and destroyed.

† All figures in the tables are means (10 plants basis) with standard deviations.

Discussion

The data in table 1 demonstrate conclusively that the effects of added CO_2 on red clover plants fixing nitrogen are due to an increase in pCO_2 rather than to an increase in the total quantity available to the plants. Indirect eivdence of this was obtained in experiments previously reported (9) in which the plants were open to the air. Theoretically, in this case, an infinite quantity of total CO_2 was available to plants in each series, but those plants in whose immediate neighborhood the pCO_2 was maintained at 0.1 to 0.2 per cent showed marked benefits in growth as compared with the controls in air.

PART II. THE EFFECT OF ADDED CARBOHYDRATES TO LEGUMINOUS PLANTS

As shown in Part I, an increase in pCO_2 under which clover plants are grown results in an increase in dry weight, nitrogen fixed, and number of nodules. These effects are believed to be caused by a higher level of carbohydrate in the treated plants. An investigation of the effect of addition of various carbohydrates to inoculated plants was therefore suggested. Laurent (6) and Mazé (8) both found increases in the yield of vetch and maize when these were supplied with carbohydrate. Knudson (5) has reported a number of experiments in which glucose, sucrose, maltose, fructose, etc., were added in concentrations from 0.1 to 2 per cent to water and agar culture solutions used for growth of leguminous and non-leguminous plants. In general, the growth of all of the plants was benefited by the addition of carbohydrate in both light and dark, and there was an increase in rate of respiration. A rather interesting observation was made that galactose was toxic to field pea, corn and wheat even in concentration as low as 0.0125 per cent; this toxicity was not evident if glucose was added with the galactose. Knudson also observed that the presence of sugar caused a marked development of pigment in corn and vetch.

The aforementioned papers deal with the influence of carbohydrates on plants supplied with combined nitrogen; of greater interest are two papers by Golding (2, 3) in which inoculated leguminous plants were used. Bean, clover, lucerne, and sanfoin were grown in sterile washed sand, (*a*) inoculated, with and without sucrose, and (*b*) supplied with NH_4NO_3 with and without sucrose. Dry weights showed an increase in the presence of the sugar in every case except the inoculated sanfoin series and the lucerne receiving NH_4NO_3. Leonard (7) added dextrose plus bacteria to a nutrient solution supplied soybeans, grown on sand in 32-ounce glass bottles plugged with cotton, in an effort to increase CO_2 inside the bottle. He reports that the dextrose had a deterimental effect on the roots of the plants, but that the nodules formed were nearly twice as large as those on the controls.

Experimental

Experiment III.—Preliminary data were obtained on the effect of adding carbohydrate to inoculated clover and alfalfa plants from two experiments not designed specifically for the investigation of this question. Golding (4) believed that the function of the leguminous plant in nitrogen fixation was to remove toxic products of growth formed by the organism. He claimed to have obtained fixation by rhizobia apart from the host plant by a method of culture which simulated the action of the plant. In a previous publication (11) we have criticized Golding's technique and conclusions, and attempted to test the idea by a method of culture which did allow the removal of soluble products of metabolism. The results were negative, but it was thought that the idea might be further examined by the following scheme. A leguminous plant, e.g., clover, was grown in the presence of a member of the rhizobia species which does not infect this plant; the organism was supplied with a source of energy external to the plant. Any soluble nitrogenous compounds secreted by the organism might be removed by the plant and thus nitrogen fixation encouraged.

With the view of testing this method or removal of soluble products by the plant, the following experiments were made. Fourteen 32-ounce bottles containing 250 cc. modified Crone's agar (N-free) were seeded with 4 plants each of red and sweet clover. Seven bottles each were inoculated with *Rh. trifolii* 209, and seven with *Rh. meliloti* 100. Three series of 14 bottles each were thus prepared; the first contained no carbohydrate; the second, 0.3 per cent mannitol; and the third, 0.3 per cent glucose.

The plants were placed in the greenhouse for 9 weeks; during this time there was a heavy growth of the bacteria on the surface of the agar. However, the plants in the presence of the non-homologous species[3] of rhizobia showed no benefit and were yellow and dead at the end of the experiment, which indicated that no soluble nitrogen compounds were being secreted by the bacteria. The red clover in the presence of *Rh. trifolii*, and sweet clover in the presence of *Rh. meliloti*, were markedly benefited, proving that the cultures did fix nitrogen in combination with the proper host plant. Analyses were made on the inoculated red clover in the presence of carbohydrate to determine whether the glucose or mannitol influenced the fixation of nitrogen. The results given in table 2, experiment III, agar series, indicate that there was an increase in the quantity of nitrogen fixed by red clover in the presence of glucose, and a slight decrease in the presence of mannitol.

The foregoing experiment was repeated except that sand plus modified Crone's solution was used as a substrate. More complete analyses were made and are given in table 2, experiment III, sand series; these confirm the results of the agar series. There is no benefit to plants grown in the presence of a non-homologous species even though the latter has a source of energy. On the other hand, the data show that both red and sweet clover inoculated with a homologous species fix more nitrogen if glucose is supplied, but mannitol has a slight depressing effect on the fixation.

Experiment IV.—In a second experiment, the red and sweet clover plants were grown in different bottles on agar, and not together as in experiment III; also mannitol only was used in this experiment. The sweet clover series did not grow well and were not analyzed. The data on the red clover series given in table 2, experiment IV, confirm the results of experiment III.

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[3] Non-homologous species—a member of a cross inoculation group that does not infect the plant under consideration.

Experiment V.—In experiments V and VI, the effect of glucose, sucrose and mannitol on the fixation of nitrogen by inoculated red clover plants was investigated. In experiment V, six red clover seedlings were planted in a 32-ounce bottle containing 250 cc. modified Crone's agar. Four concentrations of carbohydrate were used, viz., 0.05, 0.10, 0.25, and 0.50 per cent. The plants were grown for 49 days in the greenhouse (10–21–31 to 12–9–31); artificial lights were in use most of this period. At time of harvest, the plants receiving the higher concentrations of carbohydrates were red stemmed and gave some evidence of carbohydrate excess. The lengths of the plants were taken as well as dry weights and total nitrogen. The plants supplied with carbohydrates were shorter but much more stocky than the controls grown in plain agar. The data are summarized in table 3, agar series.

TABLE 2

Fixation of elemental nitrogen by plants inoculated with homologous and non-homologous species in the presence and absence of carbohydrates

TREATMENT	PLANTS ANALYZED	INOCULUM	MGM. NITROGEN PER 10 PLANTS		
			Plain	Glucose	Mannitol
Experiment III—Agar					
Red clover	Red clover	....	1.7	.....	.....
Red clover with sweet clover	Red clover	*Rh. trifolii*	10.4	15.4	8.9
Experiment III—Sand					
Red clover	Red clover	....	1.1	.....	.....
Red clover with sweet clover	Red clover	*Rh. trifolii*	26.6	27.4	20.9
Red clover with sweet clover	Red clover	*Rh. meliloti*	0.95	1.03	0.93
Sweet clover	Sweet clover	....	0.95	.....	.....
Sweet clover with red clover	Sweet clover	*Rh. trifoli*	0.63	1.08	0.75
Sweet clover with red clover	Sweet clover	*Rh. meliloti*	16.6	21.9	16.1
Experiment IV—Agar					
Red clover	Red clover	....	1.8	.....	.....
Red clover	Red clover	*Rh. trifolii*	10.1	.....	7.2
Red clover	Red clover	*Rh. meliloti*	2.4	.....	1.6

Each figure is an average of 3 to 4 determinations.

Five bottles were used in each treatment, four of which were inoculated. The nitrogen content of the uninoculated control of each treatment was very close to the mean of the plain agar controls; in order to save space, only data on the plain agar controls are given in the table. In some of the treatments it was necessary to discard one or two bottles because of mold contamination. The data in table 3 show that the addition of glucose or sucrose to Crone's agar causes an increase in both dry weight formed and nitrogen fixed by red clover plants.

The average standard deviation of the difference between the means of *nitrogen fixed* is about 0.75 mgm., therefore, an increase of 1.5 mgm. is necessary to establish definitely an increase in fixation. Likewise, an increase of about 50 mgm. in dry weight is necessary to be significant. An examination of the data shows that it is necessary to add about 0.25 per cent glucose or sucrose before the observed increases in the nitrogen fixed are beyond the limits of errors. However, the lower concentrations of these sugars show a trend toward higher fixation which might be verified with more samples. On the other hand, with only 0.10 per cent glucose and with 0.05 per cent sucrose, there is definite increase in dry weight. The *percentage nitrogen* in all treatments of the glucose and sucrose series gave variable results as shown by the relatively large standard deviation of the mean. There is some indication of a slight decrease in *percentage of nitrogen* in the presence of added carbohydrate, but this is not marked.

TABLE 3

The influence of added carbohydrate on the fixation of elemental nitrogen by clover

TREATMENT	AGAR SERIES—EXPERIMENT V				SAND SERIES—EXPERIMENT VI	
	Number of samples	Dry weight	Nitrogen		Number of samples	Total nitrogen
			Total	Per cent		
		mgm.	*mgm.*			*mgm*
No carbohydrate:						
Uninoculated.....	5	125 ± 9	1.55 ± 0.09	1.24 ± 0.17	..	....
Inoculated.......	7	199 ± 15	6.73 ± 0.49	3.38 ± 0.08	5	31.8 ± 2.0
Glucose:						
0.05 per cent.....	4	224 ± 7	7.05 ± 0.40	3.15 ± 0.14	3	28.6 ± 1.6
0.10 per cent.....	4	273 ± 12	8.37 ± 0.72	2.97 ± 0.12	4	34.4 ± 2.7
0.25 per cent.....	3	284 ± 38	9.48 ± 1.86	3.34 ± 0.33	4	33.1 ± 0.7
0.50 per cent.....	4	301 ± 10	10.00 ± 0.75	3.33 ± 0.27	4	39.3 ± 0.5
Sucrose:						
0.05 per cent.....	3	248 ± 11	7.80 ± 0.24	3.14 ± 0.06	4	32.3 ± 1.0
0.10 per cent.....	2	239 ± 10	7.50 ± 0.15	3.14 ± 0.07	4	36.4 ± 2.8
0.25 per cent.....	2	260 ± 5	9.08 ± 1.17	3.49 ± 0.42	2	39.0 ± 1.2
0.50 per cent.....	4	282 ± 7	9.02 ± 0.72	3.20 ± 0.15	4	38.8 ± 1.3
Mannitol:						
0.05 per cent.....	3	215 ± 11	6.45 ± 0.77	3.00 ± 0.11	4	29.5 ± 2.3
0.10 per cent.....	4	230 ± 22	6.95 ± 0.39	3.02 ± 0.11	4	33.3 ± 1.9
0.25 per cent.....	4	202 ± 16	6.00 ± 0.77	2.97 ± 0.24	4	15.1 ± 2.3
0.50 per cent.....	4	220 ± 11	5.82 ± 0.40	2.65 ± 0.14	4	21.5 ± 2.9

In the mannitol series, there is no indication of increased nitrogen fixation in any of the concentrations used; in the 0.50 per cent series there is a *decrease* in the nitrogen fixed. The addition of mannitol causes little change in dry weight, and the percentage of nitrogen is significantly decreased only in the 0.50 per cent series.

Experiment VI.—This was a duplicate of experiment V, using sand instead of an agar substrate. To 500 gm. of nitrogen-free sand was added 100 cc. of modified Crone's solution (nitrogen-free). The carbohydrates and mannitol were added in the required concentration from sterile 10 per cent solutions. The bottles were placed in the greenhouse on November 6, 1931; after 6 weeks they were completely filled by the plants. At this time, the cotton plugs were removed from the bottles in order to allow the plants to continue their growth; after 4 weeks of growth in the open the plants were analyzed for total nitrogen.

The data given in table 3, sand series, show that in the glucose series a significant increase in the nitrogen fixed was observed only in the 0.50 per cent series; in the sucrose series both the 0.25 and 0.50 per cent series showed significant increases in the nitrogen fixed.

In the mannitol series, there were no significant effects in the lower concentrations as judged by *milligram nitrogen fixed*, but fixation was *decreased* in the 0.25 and 0.50 per cent series. The response with mannitol was inclined to be erratic in all concentrations as is shown by the relatively large standard deviations.

Discussion

In both agar and sand, the addition of sucrose and glucose to the culture solution results in an increase in the nitrogen fixed by inoculated red clover plants. With plants grown in containers closed with a cotton plug in which the carbon assimilation is limited (9), there are indications of increased fixation with carbohydrate concentrations as low as 0.05 per cent. The increase is quite definite in concentrations of 0.10 to 0.25 per cent. With plants in open containers, the increase is noted only in concentrations of about 0.50 per cent. This is likely because of less carbohydrate starvation in these plants so that the added carbohydrates are relatively less effective. With mannitol, the results are inclined to be erratic, but there are in no case any indications of a benefit to the plant as judged by nitrogen fixation. The higher concentrations of mannitol (0.25 to 0.50 per cent) appear to exert a depressing influence on the fixation of nitrogen.

The effect of the added carbohydrate is probably twofold:

1. Activity of bacteria (rhizobia) liberates CO_2, which results in an increase in pCO_2 available to the plant. In part I of this paper, this was shown to be an important factor in nitrogen fixation by leguminous plants.

2. There is actual absorption of the carbohydrates which increases the carbohydrate level in the plant sap. That carbohydrates are actually absorbed from dilute solution by plants has been demonstrated by Knudson (5). The fact that no increase in the fixation of nitrogen took place in the presence of non-toxic concentrations of mannitol indicates that the liberation of CO_2 from the carbohydrate is not the only factor, since rhizobia can use mannitol as readily as glucose or sucrose to produce CO_2. It is of interest that the higher concentrations of mannitol appeared to exert a toxic action on the growth of clover, whereas glucose and sucrose did not.

SUMMARY

Red clover plants were supplied with the same total quantity of CO_2, but at different partial pressures. Marked increases in the dry weight formed, nitrogen fixed, and number of nodules were observed as the pressure was raised

from 0.03 per cent to 0.1 per cent. The observed increases are due to an increase in the pCO_2 supplied the plants, and not to the total quantity available.

Red clover plants supplied with glucose and sucrose in the nutriment solution show an increase in both dry weight and nitrogen fixed even in concentrations of carbohydrate as low as 0.10 per cent. However, higher concentrations of the carbohydrate are more effective. The increases are more marked in plugged containers in which carbon assimilation in the controls (no carbohydrate) is limited and in which gas diffusion is lowered. The effect is probably due to liberation of CO_2 by bacteria with consequent increase in the pCO_2 in the neighborhood of the plant, and to actual absorption of the carbohydrate.

The addition of mannitol to inoculated red clover plants does not cause an increase in nitrogen fixed. Concentrations of 0.25 to 0.50 per cent mannitol appear to have a detrimental effect on the fixation of nitrogen by red clover.

Red clover and sweet clover plants inoculated with a non-homologous species of rhizobia (one that does not infect the plant) die of nitrogen starvation even though a source of energy (carbohydrate) is furnished the bacteria. This indicates that soluble nitrogen compounds, capable of being used by the plant, are not fixed by free living forms of rhizobia.

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THE RELATION OF PHOSPHORUS TO BIOLOGICAL NITROGEN FIXATION AND THE CONFORMITY TO THE LAW OF DECREASING INCREMENT[1]

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Within recent years, there has been much interest in the development of biological methods for the determination of the fertility requirements of soils. Several of these are based upon the growth of Azotobacter, either upon soil placques (the method of Winogradsky), or by determining the amounts of nitrogen fixed in a nitrogen-free medium. The latter method is based upon the assumption that there is a direct relationship between nitrogen fixation and phosphate assimilation. Stoklasa (11) claims that 5.0 to 5.7 mgm. of nitrogen is fixed for every gram of phosphorus used, and that the minimum need of phosphorus is 2.46 mgm. (5.46 mgm. P_2O_5) per gram of glucose. These figures are usually employed in calculating the amounts of available phosphorus in soil from the quantities of nitrogen fixed.

In former articles (1, 2) the author has shown that the potassium needs of Azotobacter are very small and that there is not a direct relationship between phosphate added to Ashby's solution and the amounts of nitrogen fixed by Azotobacter.

The effects of varying amounts of fertilizer elements upon plant growth and crop yields have been studied by many investigators. As early as 1855, Liebig (3) called attention to these facts and suggested the "law of the minimum." The studies of other investigators showed, however, that increasing amounts of a fertilizer element do not produce proportional increases in crop yields, but that if yields are plotted against the amounts of the elements added (assuming the presence of sufficient amounts of all other essential elements), the resulting graph is a curve, usually of the sigmoid type, although often an exponential curve is obtained. In general, the first addition produces the greatest effect, and subsequent additions give smaller responses until there is no further increase, or, even decreased yields in some cases. Mitscherlich (5) assumed that "the increase of crop produced by unit increment of the lacking factor is proportional to the decrement from the maximum" (8), which is expressed mathematically:

$$\frac{dy}{dx} = (A - y)C$$

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[1] Contribution from the department of agricultural chemistry. The author is indebted to Dr. P. S. Burgess for permission to use unpublished results.

where y = the yield obtained when x = the amount of the factor present and A the maximum yield obtainable if the factor were present in excess. C = constant.

TABLE 1

*Effect of varying phosphate additions upon nitrogen fixation**

CULTURE	x MGM. P ADDED	y MGM. N FIXED	C	C AVERAGE	DEVIATION	AVERAGE DEVIATION
Azotobacter 3-1	0	0	0			
	0.25	1.9	0.85	0.91	+0.06	±0.05
	0.50	3.2	0.90		+0.01	
	0.75	4.0	0.98		−0.07	
	1.00	4.8	(1.69)			
	1.50	4.9				
Azotobacter 3-1	0	0	0			
	0.1	2.9	(2.20)	0.88		
	0.2	2.5	0.91		−0.03	±0.19
	0.3	3.5	0.87		+0.01	
	0.4	4.0	0.86		+0.02	
	0.6	4.2	0.63		+0.25	
	0.8	6.4	1.14		−0.26	
	1.25	7.3				
Clostridium 34 (incubated anaerobically)	0	0	0			
	0.1	2.8	(4.39)	1.66		
	0.3	2.7	1.38		+0.28	±0.18
	0.4	3.6	1.85		−0.19	
	0.6	4.0	1.74		−0.08	
	0.8	4.4				
Azotobacter 50	0	0	0			
	0.2	1.6	0.72	0.98	+0.26	±0.21
	0.3	2.5	0.83		+0.15	
	0.4	3.9	1.25		−0.27	
	0.6	4.5	1.13		−0.15	
	1.5	5.7				
Azotobacter 3-1 and Clostridium 34 (incubated aerobically)	0	0	0			
	0.25	1.3	0.50	0.55	+0.05	±0.02
	0.50	2.5	0.56		−0.01	
	0.75	3.2	0.55		0.00	
	1.00	4.5	(0.87)			
	1.50	4.5	0.58		−0.03	
	2.00	5.2				

* Unpublished data of Dr. P. S. Burgess.

Although there has been considerable controversy concerning the law of diminishing increment, it has given encouraging results in the hands of various investigators. Without regard to the various criticisms and modications of the formula given, and while Magistad, Farden, and Lambert (4) found the method of Spillman (9, 10) to be more satisfactory, the constant C ("Wirkungsfaktor") was calculated in order to determine approximately the relationship between the amounts of phosphate added and nitrogen fixed by non-symbiotic organisms.

If the unpublished results of Burgess and of Ranganathan and Norris (7) are represented graphically by plotting nitrogen fixed against phosphate added, a straight line is not obtained but a curve of the general form of an exponential function. It may be, however, that it is a sigmoid curve and that the smaller amounts of phosphate give such response that the inflection point can not be recognized. In table 1, the "Wirkungsfaktor," C, has been calculated from the data and the formula mentioned, with A being the maximum nitrogen fixed in the series to which varying amounts of phosphate had been added, y the amount of nitrogen fixed when x units of phosphorus were present. The figures enclosed in parentheses are not used in calculating averages since calculations showed they varied greatly from the mean value, and therefore their inclusion would give erroneous results.

DISCUSSION

An examination of the table reveals that although the values of C vary considerably, yet there is a fair agreement in many cases. This is especially true in the case of Azotobacter 50 and the mixed culture of Azotobacter 3-1 and Clostridium 34. In general, however, when mathematical studies have been applied to biology, the results obtained are not of the high degree of accuracy obtained in physical chemistry. An adequate explanation has not, to the author's knowledge, been advanced. Be that as it may, the results in the cases mentioned suggest that, in general, there is a fair agreement and there seems to be sufficient evidence to warrant the suggestion that the increase of nitrogen fixed by unit increment of phosphorus is proportional to the decrement from the maximum for fixation. A more striking proof is obtained when the data are represented graphically.

The values for C vary with the different cultures used, yet again the values obtained for Azotobacter 3-1 in two series of experiments and Azotobacter 50 (both strains of *A. chrococcum*) are approximately of the same order of magnitude.

Apparently, the minimum phosphate requirements for Azotobacter are very small and in most cases maximum fixation is obtained by the addition of 1 to 2 mgm. of phosphorus. Burgess has other data to show that in a series ranging from 0 to 100 mgm. P_2O_5, maximum fixation was obtained with 4 mgm. P_2O_5 (1.75 mgm. P).

Pittman and Burnham (6) have found the Winogradsky test to correlate with field observations in 70 to 80 per cent of the cases observed. Those were only in extreme ranges of phosphate content where the soil was either deficient or well supplied. In the intermediate ranges, the correlation was lower. The author believes, partly from experience and partly from observation, that the

Winogradsky test and all other tests using Azotobacter to estimate amounts of available phosphorus in soil have been most successful in those soils which are extremely deficient in phosphorus. In another article (1), evidence has been produced to show that if potassium is required by Azotobacter, the minimum requirement is extremely small. It would be expected, however, that varying amounts of potassium might have the same effect as varying amounts of phosphorus.

The results presented in this paper explain the observation of Pittman and Burnham (6) and others. If the relation of available phosphate to nitrogen fixed follows the law of decreasing increment, which is apparently the case, then small additions of phosphate to a soil deficient in phosphorus will produce a greater response than subsequent additions which may be even larger. Consequently, in the Winogradsky test, the addition of phosphate to a soil markedly deficient in phosphorus will give a better response than the addition of phosphate to a soil containing a large amount of that element.

SUMMARY

The relation between added phosphate and nitrogen fixed by Azotobacter and *Clostridium pastorianum* seems to follow the law of decreasing increment.

When the constant C ("Wirkungsfaktor") is calculated from the formula of Mitscherlich, a fair agreement is found in the results.

An explanation on this basis agrees with field observations in that the Winogradsky test is usually most accurate in soils extremely deficient in phosphate.

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COMPOSITION OF THE FOREST FLOOR LAYERS UNDER DIFFERENT FOREST TYPES ON THE SAME SOIL TYPE

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For studies of the influence of vegetation upon the composition and amount of the forest floor laid down on a uniform sandy soil, Star Island, in northern Minnesota, offers unusual advantages. It lies in Cass Lake, well within the limits of the original evergreen forest, is practically virgin, and now forms part of the Chippewa National Forest. The forest on the island is being reserved from cutting, the merchantable white and Norway pines not being harvested when mature but allowed to stand until they die or are blown down. Fires, which in the last two or three decades have destroyed or modified the forest floor and forest types over much of northern Minnesota (2), have not occurred on this island for the last 50 years. The growth rings in the callouses of the Norway and white pines and the ages of the jack pine and basswood sprouts and of the aspen suckers indicate that although there have been at least six fires upon the island during the past century and a quarter, viz.: in 1808, 1818, about 1824, 1865, 1871, and 1872, none of these was intensive enough to alter radically the composition of the forest (5).

The area of the island is somewhat less than 2 square miles and about a fifth of this is occupied by Lake Helen. The surrounding large lake, the shape of the island, the presence of this little lake, and numerous small bogs provide it with exceptional natural protection against the spread of fires. Most of it lies more than 15 feet above the surface of the water in both the surrounding lake and the enclosed lake. On this higher portion the soil is a deep uniform sand—Cass Lake fine sand (1).

The vegetation of the island was studied in considerable detail in 1915 by Pammel (6) and very recently by one of the authors (5). The principal forest types on the upland, in order of extent, are Norway pine, sugar maple-basswood, white pine, and jack pine. Their distribution is shown on a map of the island in a recent number of this journal (1, p. 282).

METHOD OF COLLECTING SAMPLES

Samples of the forest floor were gathered from representative areas of Norway pine, white pine, and sugar maple-basswood on the island on which the larger trees had been counted and measured on quarter-acre plots (5). The

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Norway pine area sampled lies near the west side of Lake Helen and that of white pine between that lake and the east shore of Star Island, whereas the maple-basswood area is on a narrow peninsula northeast of Lake Helen. The samples to represent the jack pine forest floor were not collected on the island, on which the type is limited to two small areas, because it was feared that the floor on these small tracts might not be truly representative for the jack pine type, but from a plot of the Lake States Forest Experiment Station located on the same soil type on the mainland about 3 miles south of the island. On the jack pine area over 95 per cent of the large trees were jack pine, and on the Norway pine area 98 per cent were Norway and 2 per cent white pine. On the white pine area 56 per cent were white pine and 44 per cent Norway, whereas on the maple-basswood area 58 per cent were basswood, 23 hard maple, and 19 American elm. The number per acre of the dominant trees on each of the areas and their age, height, and diameter are reported in table 1. A detailed description of the vegetation on the different areas is being published elsewhere (5).

TABLE 1

Number, age, and size of dominant trees in the different forest types

	FOREST TYPES			
	Jack pine	Norway pine	White pine	Maple-basswood
Number of large trees per acre	355	200	100	492
Age, *years*	59	200	250	60
Average height, *feet*	72	100	115	57
Average diameter, *inches*	8	17	25	7

The term *forest floor* is used in this paper, as proposed by a joint committee on forest terminology from the Society of American Foresters and the Canadian Society of Forest Engineers, "to designate only the deposits of vegetable matter on the ground in the forest." (4, p. 78), recognizing three layers—litter, duff, and leafmold. *Litter* is the upper, but slightly decomposed, portion of the forest floor, *duff*—an intermediate layer of more or less decomposed organic matter between the litter and the *leafmold or humus*—the portion in which decomposition of the litter is so far advanced that its original form is not distinguishable.

In each area selected as representative of a forest type five stations were located at intervals of 66 feet along a straight line, and samples of litter, duff, and leafmold were collected at each. In the litter only the fresh surface materials, usually very loose and showing no sign of decomposition and only slight discoloration, were included. Enough was collected from a compact area of 1 to 4 square feet to fill a sack of about 4 quarts capacity. The samples of duff and leafmold from each station represent the materials from the same area as that from which the corresponding sample of litter had been removed.

TABLE 2

Composition of freshly fallen leaves

SPECIES	PLACE AND DATE OF COLLECTION	ASH	VOLATILE MATTER	N	CaO	K_2O	P_2O_5	SO_3	pH
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Basswood (*Tilia glabra* Ventenat)	Star Island Oct. 1926	8.26	91.74	0.62	3.40	....	....	....	...
Basswood (*Tilia glabra* Ventanat)	Near Cass Lake Dec. 1931	13.12	86.88	1.31	5.43	....	0.42	....	...
Sugar maple (*Acer saccharum* Marshall)	Star Island Oct. 1926	9.07	90.93	1.82	4.14	....	....	....	...
Sugar maple (*Acer saccharum* Marshall)	Star Island Dec. 1931	15.76	84.25	0.81	3.08	....	0.23	....	...
Red oak (*Quercus borealis* Michaux f.)*	Star Island Oct. 1926	3.94	96.06	0.64	1.35	....	....	....	...
White pine (*Pinus strobus* Linnaeus)	Star Island Oct. 1926	3.61	96.39	0.42	1.21	....	....	....	...
White pine (*Pinus strobus* Linnaeus)	Star Island Oct. 1931	4.33	95.67	0.64	1.52	0.20	0.18	0.18	4.4
Maple-basswood mixture	Star Island Oct. 1931	8.44	91.56	0.84	3.95	0.50	0.50	0.28	4.5
Norway pine (*Pinus resinosa*)	Star Island Oct. 1931	4.27	95.73	0.67	1.35	0.29	0.15	0.17	4.4
Jack pine (*Pinus Banksiana*)	Near Cass Lake Oct. 1931	4.15	95.85	0.58	0.86	0.19	0.09	0.14	4.1
Jack pine (*Pinus Banksiana*)	Cloquet Oct. 1931	3.53	96.47	0.59	0.90	0.24	0.12	0.19	4.1

* The leaves of the red oak were dead but still attached to the branches.

The litter layer was about half an inch thick and the duff and leafmold layers each from one-quarter to three-quarters of an inch. In collecting the leafmold the attempt was made to avoid including material from the underlying mineral soil; such a separation was comparatively easy in the coniferous forest types, but more difficult and unsatisfactory in the maple-basswood type. Although the three layers of the forest floor are not sharply distinct but merge into one another, a very large proportion of the material in each sample is truly representative of the layer from which it was taken. At each station the three layers were removed one after the other, the whole of the material collected from a layer was well mixed, and the sample from this mixture was placed in a cloth sack and labelled before the collection from the next layer was started. All samples were air-dried within 2 or 3 days and kept in this condition until later in the laboratory each was again well mixed and then reduced to a fine state of division by means of a small hammer mill.

The samples of forest floor from the jack pine, Norway pine, and maple-basswood areas were collected during the last week of April and the first week of May, 1930, and those of white pine in July, 1931. Samples of freshly fallen leaves were collected on the island on October 9, 1926, and the analyses of these indicated the importance of analyzing samples from the areas from which the forest floor samples had been taken. Accordingly late in the fall of 1931, freshly fallen leaves were gathered close to the stations from which samples of the floor had been taken earlier; composite samples for analysis were prepared by combining equal weights from the five stations on an area. In the case of the maple-basswood two samples were collected, one in October, which was analyzed without separation of its components, and another in December, from which the maple and basswood leaves were first separated. All the data are reported in table 2.

CHEMICAL COMPOSITION

There was thus collected from Star Island and the plot of jack pine on the mainland 71 samples in all—11 of freshly fallen leaves, 20 of litter, 20 of duff, and 20 of leafmold. Determinations were made of loss on ignition, nitrogen, and lime in the case of each of the 60 individual samples of forest floor layers (table 3) but, for the determination of phosphoric acid, potash, and sulfur, composite samples were prepared by combining and thoroughly mixing equal weights of the finely ground five individual samples. Each of these 12 composite samples represents one of the three layers of the forest floor in a single forest type. In table 4 is given a summary in which the values for volatile matter, nitrogen, and lime are the averages from table 3 and those for K_2O, P_2O_5, and SO_3 are from the analysis of these composites.

Nitrogen was determined by the Kjeldahl-Gunning method and ash by prolonged ignition at dull redness in a muffle. For the other constituents reported in the tables the usual gravimetric methods were employed, an *aqua regia* extract of the ash being used. In order to avoid loss of sulfur during ignition

TABLE 3

Composition of the forest floor layers from the five stations in each of the forest types

STATION	LAYER	VOLATILE MATTER	N IN SAMPLE	CaO IN SAMPLE	VOLATILE MATTER	N IN SAMPLE	CaO IN SAMPLE
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
		Jack pine near Cass Lake			Norway pine on Star Island		
1	Litter	95.88	0.83	1.16	95.51	0.75	1.32
	Duff	82.69	1.39	1.38	92.02	1.09	1.21
	Leafmold	55.77	1.21	1.45	61.80	1.17	0.89
2	Litter	95.46	1.00	0.97	96.62	0.66	1.09
	Duff	83.70	1.33	1.07	86.73	1.23	1.47
	Leafmold	36.88	0.79	0.82	56.98	1.20	1.15
3	Litter	96.05	0.91	1.04	96.71	0.72	1.02
	Duff	79.62	1.48	1.27	83.06	1.05	1.20
	Leafmold	45.51	1.03	1.14	54.35	0.99	0.84
4	Litter	93.92	0.85	1.26	97.09	0.72	0.93
	Duff	81.99	1.28	1.15	93.84	1.00	0.99
	Leafmold	42.64	0.87	1.16	52.86	0.86	0.69
5	Litter	96.28	0.74	0.84	97.09	0.58	0.84
	Duff	87.12	1.29	0.92	85.12	1.37	1.35
	Leafmold	56.65	0.98	0.69	62.39	1.11	0.66
Average....	Litter	95.52	0.87	1.05	96.60	0.69	1 04
	Duff	83.02	1.35	1.16	88.15	1.15	1.24
	Leafmold	47.49	0.98	1.05	57.68	1.07	0.85
		White pine on Star Island			Sugar maple-basswood on Star Island		
1	Litter	92.83	0.98	1.44	86.53	1.15	4.16
	Duff	82.21	1.59	2.19	80.71	1.85	5.26
	Leafmold	52.66	1.43	1.91	57.32	1.90	4.04
2	Litter	92.64	1.02	1.48	87.30	1.21	4.70
	Duff	75.90	1.33	1.83	79.51	2.04	5.52
	Leafmold	54.64	1.24	1.66	50.57	1.86	4.12
3	Litter	93.12	0.90	1.28	86.13	1.16	4.81
	Duff	75.39	1.31	1.50	79.93	2.01	5.65
	Leafmold	49.50	1.23	1.16	40.04	1.43	2.79
4	Litter	94.60	0.80	1.25	90.25	1.06	4.54
	Duff	89.10	1.32	1.69	84.94	1.85	5.30
	Leafmold	66.85	1.61	1.28	60.04	2.29	4.65
5	Litter	92.56	0.99	1.59	90.48	1.26	4.35
	Duff	85.75	1.50	2.11	86.76	1.88	5.63
	Leafmold	59.56	1.54	2.03	47.35	1.62	3.62
Average....	Litter	93.15	0.94	1.41	88.14	1.17	4.51
	Duff	81.67	1.41	1.86	82.37	1.93	5.47
	Leafmold	56.64	1.41	1.61	51.07	1.82	3.84

the samples were first moistened with a solution of magnesium nitrate and then heated until oxidation of the organic matter was complete.

In some of the properties of the forest floor differences between the forest types are negligible, but in others they are striking. The range of variation is widest in the percentage of lime and next in that of nitrogen.

TABLE 4

Composition of freshly fallen leaves and forest floor

FOREST TYPE	FRESHLY FALLEN LEAVES	LITTER	DUFF	LEAF-MOLD	FRESHLY FALLEN LEAVES	LITTER	DUFF	LEAF-MOLD
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
	Ash				Volatile matter			
Jack pine	4.15	4.48	16.98	52.51	95.85	95.52	83.02	47.49
Norway pine	4.27	3.40	11.85	42.32	95.73	96.60	88.15	57.68
White pine	4.33	6.85	18.33	43.36	95.67	93.15	81.67	56.64
Maple-basswood	8.44	11.86	17.63	48.93	91.56	88.14	82.37	51.07
	Lime in sample				Lime computed to organic matter			
Jack pine	0.86	1.05	1.16	1.05	0.90	1.10	1.40	2.20
Norway pine	1.35	1.04	1.24	0.85	1.41	1.08	1.42	1.48
White pine	1.52	1.41	1.86	1.61	1.59	1.51	2.28	2.87
Maple-basswood	3.95	4.51	5.47	3.84	4.31	5.12	6.65	7.51
	Nitrogen in sample				Nitrogen in organic matter			
Jack pine	0.58	0.87	1.35	0.98	0.61	0.90	1.71	2.12
Norway pine	0.67	0.69	1.15	1.07	0.70	0.73	1.30	1.84
White pine	0.64	0.94	1.41	1.41	0.67	1.01	1.73	2.49
Maple-basswood	0.84	1.17	1.93	1.82	0.92	1.33	2.36	3.56
	P_2O_5 in sample				K_2O in sample			
Jack pine	0.09	0.16	0.22	0.15	0.19	0.14	0.18	0.14
Norway pine	0.15	0.14	0.22	0.22	0.29	0.20	0.19	0.19
White pine	0.18	0.29	0.23	0.23	0.20	0.23	0.22	0.24
Maple-basswood	0.50	0.35	0.38	0.36	0.50	0.32	0.36	0.46
	SO_3 in sample							
Jack pine	0.14	0.19	0.30	0.23				
Norway pine	0.17	0.19	0.26	0.27				
White pine	0.18	0.19	0.29	0.22				
Maple-basswood	0.28	0.29	0.46	0.48				

Volatile matter and ash

With all four types the content of volatile matter decreases from the freshly fallen leaves, with 92 to 96 per cent, to the leafmold, with only 47 to 58 per cent, while the ash increases correspondingly. A greater difference was found between duff and leafmold than between litter and duff; in the case of the former two the minimum was with the white pine, 25 per cent, and the maximum with the jack pine, 36 per cent. Between the freshly fallen leaves and the litter the difference was less than between litter and duff, ranging from less than 1 per cent to 4 per cent. A comparison of the volatile matter of each of the three layers of forest floor from the 20 individual stations shows that the only characteristic difference between the different forest types appears to be that all the samples of maple-basswood litter carry much more ash than the litter from the pine types, among which the Norway has the least. Those from the white pine lie between the jack pine and maple-basswood.

Lime

Between the jack pine and Norway pine types the differences are small and probably not significant. The marked differences are between either of these and the maple-basswood, in which the lime content is almost five times as high in each of the three layers, whether it be expressed as the percentage of the dry sample or of only the volatile matter. Expressed in the latter form, it is only 1.08 per cent in the Norway pine litter but 5.12 per cent in that of the maple-basswood. The corresponding percentages for the leafmold are 1.48 and 7.51, and those for the duff are intermediate. The values for the three layers of the white pine are between those for the Norway pine and the maple-basswood but decidedly nearer the former. It seems probable that the differences between white pine and Norway or jack pine are caused, at least largely, by the greater admixture of the leaves of sugar maple and other deciduous plants rather than by any characteristic properties of the white pine itself.

Although no regular difference in the lime content of the dry samples of the three layers is shown it appears in all cases when we compare the percentages based upon the volatile matter. It is lowest in the litter and highest in the leafmold.

Nitrogen

Although the greater amounts of nitrogen in the maple-basswood floor are not so striking as those of lime, they may still have considerable significance in the maintenance and improvement of the deciduous as compared with the coniferous forest soils. The content of nitrogen in all three layers of the maple-basswood, whether expressed as the percentage of the dry sample or as that of the organic matter, is from one and one-half times to twice as high as in the jack pine and Norway pine types. The values for the litter are 1.33 and 0.73 per cent respectively and for the leafmold 3.56 and 1.84 per cent. The layers of the white pine have intermediate values. The freshly fallen needles of the white pine are slightly lower in nitrogen content than those of Norway pine.

With all the individual sets of all four types there is an increase in the nitrogen content of the organic matter from the litter to the leafmold. As all the nitrogen is contained in the organic matter, reported in the tables as *volatile matter*, any admixture of mineral soil with the plant remains will lower the the percentage of nitrogen in the sample. Hence, significant differences are to be sought in the nitrogen content of the organic matter rather than in that of the dry samples.

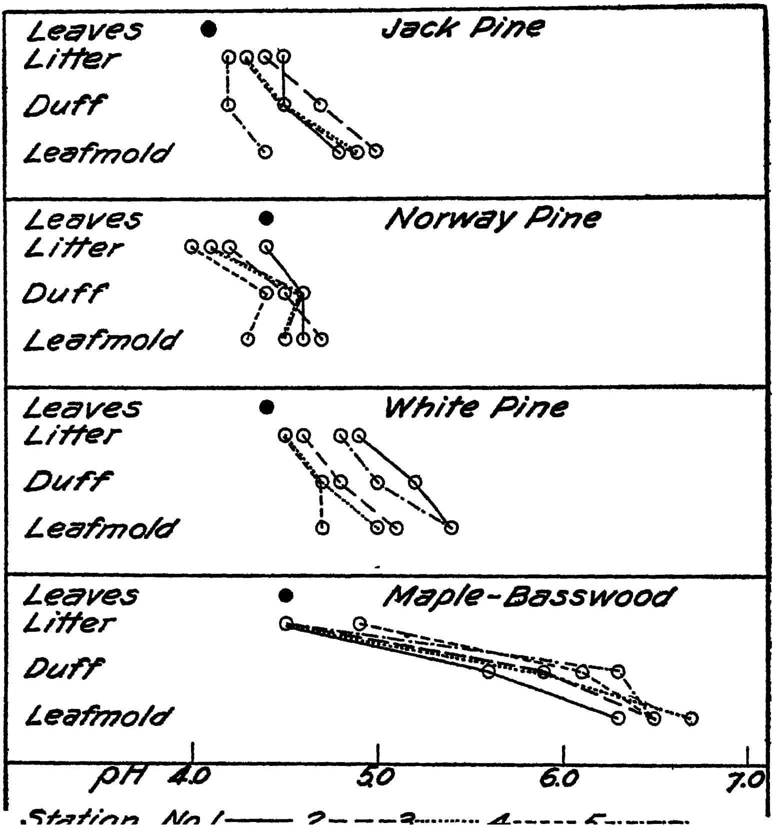

FIG. 1. HYDROGEN-ION CONCENTRATION OF THE FOREST FLOOR LAYERS ON DIFFERENT FOREST TYPES

Five sets of the forest floor layers were taken on each type, but only one sample of freshly fallen leaves.

Phosphoric acid, potash, and sulfur

Phosphoric acid, potash, and sulfur were much higher in the three layers of the maple-basswood forest floor than in those from under the pines.

ACIDITY

The pH value was determined on all the individual samples after grinding, using the gas chain electrometric method, with a bubbling hydrogen electrode and constant mechanical shaker. The values for the individual samples are shown in figure 1. In general the acidity varies inversely as the lime content. In the litter layer the differences between the forest types are small, the Norway pine on the whole being a little more acid than the jack pine, which in turn is a little more acid than the white pine or the maple-basswood, the last two being much alike (table 5). In the case of the Norway pine four of the litter samples were more acid than the freshly fallen leaves and the fifth was of the same degree of acidity, whereas with the jack pine and white pine none of the litter samples was as acid as the corresponding leaves; with the maple-basswood four were just as acid and the fifth somewhat less.

The duff layer shows greater differences. All the duff samples from the maple-basswood are far less acid than any of the corresponding litter samples and each is at least considerably more acid than its accompanying leafmold; so while the leaves and the litter are strongly acid the duff is only moderately acid and the leafmold is almost neutral. In the case of the white pine also there is a decrease in acidity from the litter to the leafmold, the duff occuping an intermediate position except in one set, in which the acidity is alike in duff and leafmold. Although the white pine forest floor resembles that of the maple-basswood in showing a decrease in acidity on proceeding from the surface downward, the change in the case of the former is far less rapid and the acidity of both leafmold and duff is in all cases higher than that of the duff of the maple-basswood.

TABLE 5

pH values of forest floor layers under different forest types

	LITTER			DUFF			LEAFMOLD		
	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Jack pine	4.2	4.5	4.3	4.2	4.7	4.5	4.4	5.0	4.9
Norway pine	4.0	4.4	4.1	4.4	4.6	4.6	4.3	4.7	4.5
White pine	4.5	4.9	4.6	4.7	5.2	4.8	4.7	5.4	5.1
Maple-basswood	4.5	4.9	4.5	5.6	6 3	5.9	6.3	6.7	6.5

In the acidity of its forest floor the jack pine more closely resembles the white pine than does the Norway, but there is not the same regular decrease with depth, in two of the five sets the duff being found as acid as the litter. Although the needles of the Norway pine were not as strongly acid as those of the jack pine its forest floor is in general more acid, especially the leafmold. Unlike the other forest types the Norway pine in one set shows as high an acidity in the leafmold as in the duff and in three of the four other sets a higher acidity.

INFLUENCE OF THE FOREST TYPE UPON THE COMPOSITION OF THE FOREST FLOOR

From the tables it is very evident that the presence of maple or basswood trees greatly increases the content of lime in all three layers of the underlying forest floor. The percentages of lime in all the samples from the maple-basswood area are between 3.08 and 5.43 per cent whereas in those from the jack and Norway pine areas they are below 1.40 per cent. Between the freshly fallen leaves and those which have been exposed to the weather for 6 months or longer and constitute the *litter* of the present study there are no great differences in the percentage of lime. In the duff and leafmold the percentage, computed to the organic matter, is successively higher. The question whether the floor of the Norway pine is generally poorer in lime than that of the jack pine must be regarded as not definitely answered by the data of this study although these and also those obtained in an earlier study, in which the needles were allowed to fall on a canvas but were left exposed to the weather until gathered at the end of a year (3, p. 718), suggest an answer in the affirmative. Also it is not certain that the white pine needles are regularly richer in lime than those of the jack pine, or that the underlying floor would be richer if it did not receive additions of lime from hardwood leaves. In all the samples of litter and duff of the white pine floor on Star Island there was a considerable proportion derived from hardwoods while the samples from the jack and Norway pine floors were practically free of hardwood leaves.

The hydrogen-ion concentration is not closely correlated with the lime content, for it is almost as high in the freshly fallen leaves and litter of the maple-basswood as in the corresponding samples from the pines, although far lower in the duff and leafmold. In these lower layers of the white pine floor it is somewhat lower than in those of the jack and Norway pines; the difference may be chiefly or entirely the result of the influence of the hardwood leaves.

The addition of maple and basswood leaves raises the lime content of all the layers of the forest floor markedly, increases the nitrogen content distinctly, and greatly lessens the acidity of the duff and leafmold, thus rendering the site much more favorable for plants sensitive to acidity or requiring a liberal supply of available lime.

FOREST FLOOR OF JACK PINE IN CLOQUET STATE FOREST

From a 60-year-old stand of jack pine in the Cloquet State Forest, which is about 110 miles east of Cass Lake, samples of forest floor were collected at the end of June, 1930, by the method described. Samples of litter, duff, and leafmold were taken from five stations about 66 feet apart. The data are given in table 6. The soil of this part of the state forest, the northeast corner of Sec. 30, Township 49, Range 18 West, has been mapped as Omega loamy sand.

The litter and duff are similar in composition to those gathered at Cass Lake except that they show lower percentages of lime. The leafmold samples show greater differences, those from Cloquet having a much lower pH, a lower percentage of nitrogen, and about half as much lime.

TABLE 6

Composition and acidity of forest floor of jack pine near Cloquet, Minnesota

STATION	LAYER	VOLATILE MATTER	N IN SAMPLE	N IN ORGANIC MATTER	CaO IN SAMPLE	CaO COMPUTED TO ORGANIC MATTER	pH VALUE
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	
1	Litter	94.27	0.92	0.97	0.82	0.87	4.5
	Duff	89.32	1.35	1.51	0.76	0.85	4.5
	Leafmold	63.82	1.02	1.59	0.54	0.85	4.3
2	Litter	92.11	0.89	0.96	0.86	0.93	4.4
	Duff	83.34	1.35	1.61	0.84	1.01	4.4
	Leafmold	54.76	0.87	1.69	0.74	1.35	4.3
3	Litter	92.62	1.24	1.34	0.92	0.99	4.4
	Duff	81.33	1.56	1.91	0.81	1.00	4.1
	Leafmold	47.95	0.83	1.73	0.64	1.34	4.3
4	Litter	93.85	0.93	0.99	0.59	0.63	3.9
	Duff	88.12	1.43	1.62	0.58	0.66	4.0
	Leafmold	63.04	0.84	1.33	0.46	0.73	3.9
5	Litter	92.57	0.86	0.92	0.77	0.83	4.9
	Duff	89.38	1.28	1.43	0.69	0.77	4.0
	Leafmold	55.42	0.85	1.53	0.60	1.08	4.2
Average....	Litter	93.06	1.02	1.09	0.79	0.85	...
	Duff	86.30	1.39	1.62	0.74	0.86	...
	Leafmold	57.00	0.88	1.55	0.60	1.07	...

SUMMARY AND CONCLUSIONS

Litter, duff, leafmold, and freshly fallen leaves were collected from representative areas of Norway pine, white pine, and sugar maple-basswood forest on Star Island, Minnesota, and from a nearby jack pine area, all on the same sandy soil type. There is evidence that fire had not appreciably affected the forest floor on any of the areas for at least 60 years. Determinations of ash, lime, nitrogen, and acidity were made.

With all four types a progressive increase is found in the percentages of ash, lime, and nitrogen, together with a decrease in acidity, on passing from the litter to the duff and from the duff to the leafmold.

The differences between the jack pine and the Norway pine types are small, but between these and the maple-basswood type they are marked. These differences are to be attributed to the influence of the respective forest types and their associated vegetation, as the mineral soil under all four forest types is similar.

In the maple-basswood area the litter, duff and leafmold each carry about five times the percentage of lime and one and a half times to twice as much nitrogen as is found on the jack and Norway pine areas. In the white pine floor the values for lime and nitrogen in each of the layers lie between those for the Norway or jack pine and the maple-basswood, but decidedly nearer the former.

In acidity, differences between forest types are negligible in the freshly fallen leaves and small in the litter, but the duff and leafmold of the maple-basswood are far less acid than the same layers of the Norway and jack pine while the white pine occupies an intermediate position. Although in general the acidity of the three forest floor layers varies inversely as the lime content, with the maple-basswood, in passing from litter to duff and from duff to leafmold, the acidity decreases much more rapidly than the lime increases. In contrast the maple-basswood leaves are about as acid as the needles of the pines although they are about three times as rich in lime.

Phosphoric acid, potash, and sulfur are much the highest in the layers of the hardwood forest.

The freshly fallen leaves are noticeably similar in composition to those of the underlying litter. Those of the maple and basswood contain much more ash than, and twice as much lime as, the pine needles but are not much richer in nitrogen. The leaves of white pine are similar in composition to those of the Norway pine.

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DISTRIBUTION OF VOLATILE MATTER, LIME, AND NITROGEN AMONG LITTER, DUFF, AND LEAFMOLD UNDER DIFFERENT FOREST TYPES

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A recent article (1) reported the composition of the litter, duff, and leafmold collected from four forest types in northern Minnesota—maple-basswood, white pine, Norway pine, and jack pine—but not the absolute or relative amounts of the floor and its constituents. In order to obtain such data, samples were collected differently in June, 1932, from the same four areas, viz: maple-basswood, white pine, and Norway pine on Star Island and jack pine on the mainland nearby. Although none of the sites of the first sampling was found, it is probable they were very close to where the new samples were taken. In addition a set was taken from the smaller jack pine area on Star Island (2, p. 282), the large trees on this being older—100 years—, taller—86 feet—, of greater diameter—11 inches—, and forming a thinner stand—260 per acre—than on the jack pine area sampled on the mainland. The corresponding values on the latter were 59, 72, 8 and 355, respectively.

METHOD OF SAMPLING

On each of the five areas five stations were located at sites representative of the forest type. On the white pine area the stations were at irregular distances, the site of each being selected so as to have it the center of a group of large white pines. On the other areas the stations were either at intervals of 66 feet in a straight line, or located as the corners of a pentagon, except where a rigid adherence to this rule would have rendered the site unrepresentative for the forest type. At each of these 25 stations 5 samples were removed, viz: living undergrowth, litter, duff, leafmold, and surface 2.75-inch layer of mineral soil. On each area the five individual samples from a layer, each from exactly 1 square foot, were placed in a sack as a composite sample. A square steel form, 12 inches by 12 inches inside measurement, 2.75 inches high and provided with an inside cutting edge, was placed on the surface and forced down to the mineral soil. First the undergrowth, consisting of herbaceous and woody plants, was pulled up, the roots generally breaking off near the surface of the leafmold, then the litter was removed, next the duff, and lastly the leafmold. The separation of the last was somewhat arbitrary, as the surface of the mineral soil was rough and more or less pitted and there was no sharp line of demarcation between leafmold and the loamy surface of the mineral soil, especially in the maple-basswood type. It was sought to include in the leafmold sample all of the leafmold, so as to learn the total amount of the organic matter and nitrogen per unit area, even at the risk of affecting the percentage composition through inclusion of surface soil. In this respect the method of collection differed from that described in the earlier paper; there the aim was to exclude the mineral soil as completely as possible, even at the risk of leaving out part of the leafmold. After the forest floor had been removed from within it, the steel square was driven down flush with the surface of the mineral soil, and then the enclosed soil was taken out with a small plasterer's trowel down to the level of the cutting edge.

In order that there might be sufficient material for analysis of the undergrowth a second sample of this was collected on each area, from two 1-foot squares adjacent to each of the five stations. The weight of undergrowth per acre reported in the tables is based upon what was gathered from the five 1-foot squares, and the analyses were made of the latter combined with the sample from the adjacent 10 square feet.

In the case of the forest floor layers and of the undergrowth, the entire samples were brought to the laboratory, dried, weighed, ground in a small hammer mill, and thoroughly mixed. The composites of mineral soil were weighed in the forest after being thoroughly mixed, and a weighed sample was brought to the laboratory for moisture determination and analysis.

Nitrogen was determined by the Kjeldahl-Gunning method, volatile matter by prolonged ignition to dull redness in a muffle, and lime by extraction of the resulting ash with *aqua regia* and precipitation from the extract of the calcium in the form of oxalate.

COMPOSITION AND WEIGHT OF FOREST FLOOR LAYERS

The percentages of volatile matter, lime, and nitrogen, reported in table 1, are very similar to those of the earlier collection from the same areas (1). The differences in volatile matter are small in the case of the litter but much larger with the duff of all three pines and with the leafmold of the Norway and white pine, suggesting that in this collection more of the doubtful zones between duff and leafmold and between leafmold and mineral soil had been included in the upper of the two layers. The pH values of the corresponding layers were almost the same as in the first collection.

The white pine floor carries the largest amount of dry matter, about 35 tons per acre, and the jack pine area on Star Island the least (table 2). On the maple-basswood area there are the least litter and the least duff but the most leafmold, the amounts per acre being about 1 ton, 4 tons, and 25 tons, respectively. The white pine has almost as heavy a layer of leafmold, 20 tons, and a much heavier duff layer, 12 tons. The data do not suggest significant differences between the jack and Norway pines, which have 8 to 10 tons per acre of leafmold, 6 to 10 tons of duff, and 1.5 to 3 tons of litter.

The relative amounts of volatile matter are in general like those of dry matter. On the white pine area the leafmold carries only 44 per cent of the total volatile matter of the forest floor whereas on the maple-basswood area it carries 76 per cent (table 3).

TABLE 1

Composition of forest floor layers

LAYER	JACK PINE		NORWAY PINE	WHITE PINE	MAPLE-BASSWOOD
	Mainland	Island			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Volatile matter					
Litter	91.28	87.17	94.60	93.04	85.54
Duff	74.84	69.22	75.06	78.98	79.88
Leafmold	39.16	32.27	31.03	44.34	53.07
Entire floor	60.34	53.48	55.75	59.23	57.84
Lime in sample					
Litter	1.07	1.06	1.01	1.47	3.86
Duff	1.08	0.79	1.07	1.57	4.45
Leafmold	0.76	0.32	0.49	1.03	3.42
Entire floor	0.93	0.60	0.77	1.25	3.58
Lime computed to volatile matter					
Litter	1.17	1.22	1.07	1.58	4.51
Duff	1.44	1.14	1.42	1.99	5.56
Leafmold	1.94	0.99	1.58	2.32	6.44
Entire floor	1.54	1.12	1.37	2.11	6.18
Nitrogen in sample					
Litter	0.98	1.15	0.81	1.13	1.50
Duff	1.17	1.09	1.12	1.38	1.82
Leafmold	0.84	0.54	0.57	0.88	1.58
Entire floor	1.00	0.82	0.80	1.07	1.61
Nitrogen in organic matter					
Litter	1.07	1.32	0.86	1.21	1.75
Duff	1.56	1.57	1.47	1.75	2.28
Leafmold	2.14	1.67	1.84	1.99	2.98
Entire floor	1.66	1.54	1.42	1.81	2.78

Much the largest amount of lime is found in the floor of the maple-basswood, 2,139 pounds per acre of CaO. There is only two-fifths as much in the white pine and from a tenth to a fifth as much on the jack and Norway pine areas. Of the entire amount of lime in the forest floor the leafmold carries about four-fifths on the maple-basswood area, one-half on the white pine but only about one-third on the jack and Norway pine areas.

In nitrogen also the floor of the maple-basswood is richer than that of the pines, carrying 963 pounds per acre, about one third more than under the white pines and two or three times as much as under the other pines. About four-fifths of the nitrogen of the maple-basswood floor is in the leafmold.

TABLE 2

Weight per acre of oven-dry matter in forest floor and of its constituents

LAYER	JACK PINE		NORWAY PINE	WHITE PINE	MAPLE-BASSWOOD
	Mainland	Island			
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
		Dry matter			
Litter	3,111	5,376	5,875	3,476	2,037
Duff	19,570	11,215	12,640	24,970	8,200
Leafmold	19,330	16,860	19,089	40,910	49,550
Entire floor	42,011	33,451	37,604	69,356	59,787
		Volatile matter			
Litter	2,840	4,685	5,558	3,233	1,742
Duff	14,940	7,764	9,485	19,720	6,552
Leafmold	7,566	5,444	5,937	18,140	26,300
Entire floor	25,346	17,893	20,980	41,093	34,594
		Lime			
Litter	33	57	59	51	79
Duff	211	89	135	392	365
Leafmold	147	54	94	422	1,695
Entire floor	391	200	288	865	2,139
		Nitrogen			
Litter	31	62	48	39	31
Duff	229	122	142	345	149
Leafmold	162	91	109	360	783
Entire floor	422	275	299	744	963

The content of P_2O_5 in the maple-basswood forest floor layers was determined by extracting the ash with *aqua regia.* The percentages are: litter—0.32, duff—0.30, and leafmold—0.33. The corresponding amounts per acre would be 6.5, 24.6, and 163.5 pounds, respectively. This gives a total of 194.6 pounds per acre for the entire forest floor, or the equivalent of a little more than 1,200 pounds of 16 per cent superphosphate.

AMOUNT AND COMPOSITION OF UNDERGROWTH

No attempt was made to separate the different species in the undergrowth before grinding the samples, for we were interested to learn only whether the lime and nitrogen content of the undergrowth on the different areas showed differences corresponding to those in the forest floor. No such striking differences were found (table 4). The lime is only a little higher on the maple-

TABLE 3

Percentage distribution of lime, nitrogen, and volatile matter among the different layers of the forest floor

LAYER	JACK PINE		NORWAY PINE	WHITE PINE	MAPLE-BASSWOOD
	Mainland	Island			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
			Lime		
Litter	8	28	20	6	4
Duff	54	45	47	45	17
Leafmold	38	27	33	49	79
			Nitrogen		
Litter	7	23	16	5	3
Duff	54	44	48	46	16
Leafmold	39	33	36	49	81
			Volatile matter		
Litter	11	26	27	8	5
Duff	59	43	45	48	19
Leafmold	30	31	28	44	76

TABLE 4

Composition and weight per acre of undergrowth

COMPOSITION	JACK PINE		NORWAY PINE	WHITE PINE	MAPLE-BASSWOOD
	Mainland	Island			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Ash	7.13	6.07	5.85	7.95	9.62
Volatile matter	92.87	93.93	94.15	92.05	90.38
Lime	1.12	0.93	0.82	1.08	1.29
Nitrogen	1.42	1.02	1.23	1.88	1.82
WEIGHT PER ACRE	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Dry matter	1,172	864	883	154	269
Lime	13	8	7	2	3
Nitrogen	17	9	11	3	5

basswood area than under the pines, 1.29 per cent compared with 0.82 to 1.12 per cent. With the nitrogen the difference is somewhat greater, being higher under the maple-basswood and white pines than under the jack and Norway pines.

The dry weight of the undergrowth is greatest under the jack and Norway pines, 864 to 1,172 pounds per acre and least under the white pines, 154 pounds. Its lime content ranged only from 2 to 13 pounds per acre and the nitrogen from 3 to 17 pounds, amounts so small compared with those in the forest floor that it would make little difference in the results if the undergrowth were included with the forest floor in sampling (table 5).

TABLE 5

Relative amounts of lime and nitrogen in undergrowth and forest floor

Amount in forest floor = 100

	JACK PINE		NORWAY PINE	WHITE PINE	MAPLE-BASSWOOD
	Mainland	Island			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Lime	3.3	4.0	2.4	0.2	0.1
Nitrogen	4.0	3.3	3.7	0.4	0.5

TABLE 6

Lime and nitrogen content of surface soil

	JACK PINE		NORWAY PINE	WHITE PINE	MAPLE-BASSWOOD
	Mainland	Island			
pH	5.5	4.9	5.0	5.1	5.7
Lime (CaO), *per cent*	0.22	0.11	0.09	0.18	0.39
Nitrogen, *per cent*	0.09	0.06	0.09	0.10	0.25
Volatile matter, *per cent*	3.76	3.54	4.91	4.94	8.20
Weight of 2.75 inch layer, *tons per acre*	334	370	320	331	318
Weight of lime in layer, *pounds per acre*	1,470	814	576	1,193	2,479
Weight of nitrogen in layer, *pounds per acre*	568	429	544	663	1,589

LIME AND NITROGEN IN SURFACE SOIL

The amounts of nitrogen and lime in the surface soil compared with those in the three layers of the forest floor are shown in figure 1. Although the percentage of nitrogen in the forest floor is from 7 to 19 times as high as that in the surface of the underlying mineral soil the amount per unit area is usually smaller (table 6). Only on the white pine area is there more in the forest floor than in the surface 2.75 inches of soil (table 7). The low volatile matter percentages, 3.5 to 5.0 under the pines and 8.2 under the maple-basswood, indicate that the leafmold had been well separated and so does not contribute any important part of the nitrogen recorded for the surface soil.

The lime soluble in *aqua regia* was determined by the same method as that used with the forest floor in order to obtain comparable data. Only on the maple-basswood area does the floor carry nearly as much per acre as the surface soil; with the Norway and jack pines it carries only half as much or less. If the forest floor were burned and the resulting ash incorporated with the surface few inches of soil it may be assumed that the readily available lime would be slightly more than doubled on the maple-basswood area, be raised by 70 per cent on the white pine, and by 25 to 50 per cent on the jack and Norway pine areas.

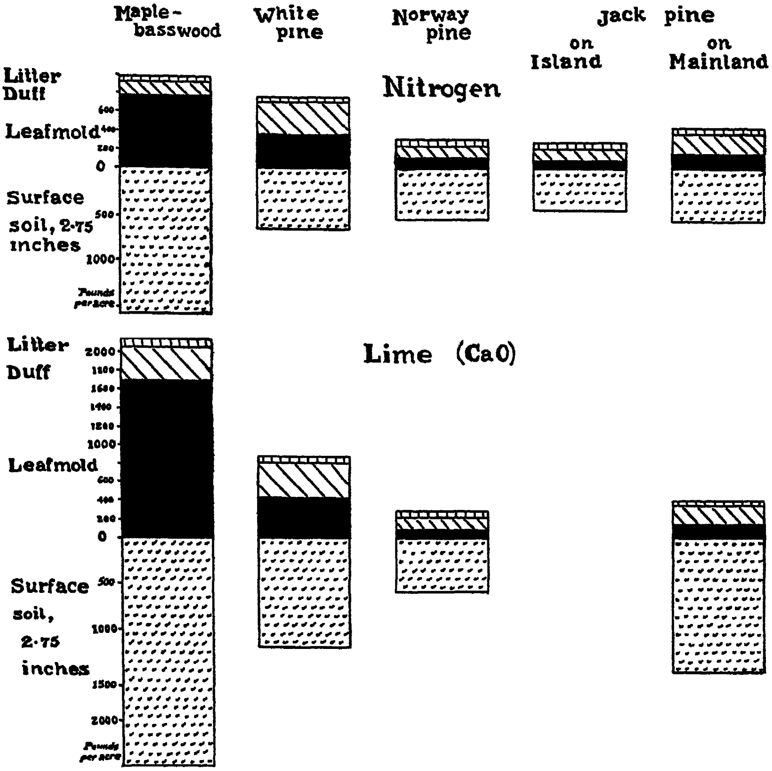

FIG. 1. NITROGEN AND LIME UNDER DIFFERENT FOREST TYPES

The distribution among the three layers of the forest floor and the underlying 2.75 inches of mineral soil is shown in the form of pounds per acre.

AMOUNTS OF LIME AND NITROGEN IN ANNUAL LEAF FALL

On October 17, 1932, when it was estimated that less than 5 per cent of the leaves were still attached to the trees, chiefly the young maples, the freshly fallen leaves, together with the maple and basswood seeds, were gathered from five units of 4 square feet each, 66 feet apart on both the southwest and the southeast maple-basswood areas on Star Island. Several weeks before, sharp frosts had occurred and very little rain had fallen since. Thus the separation from the more or less weathered leaves of the litter layer was very easy. The dry matter per acre averaged 1,997 pounds, containing 3.69 per cent CaO and 1.14 per cent nitrogen. Allowing for the leaves that had not yet fallen, this would give 78 pounds of lime and 24 pounds of nitrogen in the annual fall of leaves, to which should be added small but undetermined amounts for twigs and bark and for leaves that had fallen during the summer and were not included but were left with the litter. These amounts per acre are practically the same as those found for the maple-basswood litter collected 4 months earlier (table 2)—79 pounds of lime and 31 of nitrogen.

TABLE 7

Comparison of weights of lime and nitrogen in forest floor and underlying 2.75 inches of soil

	JACK PINE		NORWAY PINE	WHITE PINE	MAPLE-BASSWOOD
	Mainland	Island			
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
		Lime per acre			
Forest floor	391	200	288	865	2,139
Surface soil	1,470	814	576	1,193	2,479
		Nitrogen per acre			
Forest floor	422	275	299	744	963
Surface soil	568	429	544	663	1589
		Relative amounts in forest floor*			
Lime	27	25	50	73	86
Nitrogen	74	64	55	112	61

* Amount in surface soil = 100.

No collection of the annual fall of pine needles has been made on Star Island but data have been reported for forests of jack pine and of mixed Norway and white pine on the nearby mainland (3), averaging about 1,800 pounds of dry matter containing 16 pounds of lime and 17 pounds of nitrogen per acre, from one-fourth to one-half the quantities found in the litter layer.

SUMMARY AND CONCLUSIONS

From representative areas of four forest types, jack pine, Norway pine, white pine, and sugar maple-basswood, all on the same soil type, Cass Lake fine sand, on Star Island, Minnesota, litter, duff, leafmold, surface soil, and living undergrowth were collected for the determination of the weights per unit area of volatile matter, lime, and nitrogen.

The maple-basswood had the least litter and the least duff but the most leafmold, about 1 ton, 4 tons, and 25 tons per acre, respectively, of oven-dry matter. The white pine had more duff, but less leafmold, whereas the Norway and jack pines were similar, having a smaller total amount with a still lower proportion of this in the leafmold. The relative amounts of volatile matter were much the same as those of dry matter.

The largest amounts of both lime and nitrogen were found in the forest floor of the maple-basswood, 2,139 pounds per acre of CaO and 963 of nitrogen. The white pine was next highest, with 40 per cent as much lime and 75 per cent as much nitrogen, whereas the jack and Norway pine floors were lower, carrying only 10 to 20 per cent as much lime and 22 to 45 per cent as much nitrogen.

The percentage of nitrogen in the surface 2.75 inches of the underlying mineral soil was only from one-seventh to one-nineteenth as high as that in the forest floor but the weight per unit area was in all cases greater except under the white pine. The weight of lime per unit area soluble in *aqua regia* was greater in this surface layer than in the forest floor, in the case of all the forest types, that on the jack pine areas being four times as high.

The percentages of lime and nitrogen in the undergrowth of the different forest types were much alike, and the amount in each ranged only from 2 to 17 pounds per acre.

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ERRATUM

Studies on readily soluble phosphate in soils: I.

By H. W. Lohse and G. N. Ruhnke, SOIL SCIENCE, Vol. XXXV

Page 447: The ordinate of figure 2, *Mgm. P in 100 cc. Leachate*, should read *Mgm. P Leached*.

SYMBIOTIC NITROGEN FIXATION IN THE GENUS *CASUARINA*

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Several species of the genus *Casuarina* are included among the comparatively few non-leguminous plants noted as having nodules on their roots. Despite considerable research, apparently nothing has been published on work with the trees themselves showing conclusively that in this genus a relation exists between the causal organism of the nodule and the host like that existing between the nitrogen-fixing bacteria and the legumes. This paper deals with certain phases of investigations with several *Casuarina* species, initiated in the summer of 1930, with reference mainly to the ability of the causal organism to fix atmospheric nitrogen and the effect on the host.

HISTORICAL REVIEW

Among the first to report observations of the nodule-like growths on the roots of *Casuarina* trees were Schimper (13) in 1891, Janse (5) in 1897, and Barber (1) in 1903, who noted their presence but apparently made no complete histological studies nor extended investigations to determine definitely their cause or their effect on the host. Janse thought the cause to be a fungus and suggested the possibility of a symbiotic relation, whereas Barber stated that the roots of *Casuarina* trees in Bangalore were badly affected with a parasite resembling alder root tubercles.

Cavarra (2), in 1893, referred to a species of *Hymenogaster* as a parasitic fungus infecting the roots of *Casuarina*, but his work probably was confined to the roots only and had no connection with the nodular growths.

Kamerling (6), in 1911, reported the presence of the root growths, which he called "nodules," on *C. equisetifolia* trees in Java. He found bodies in fresh nodule tissue that resembled the bacteroids of the nitrogen-fixing bacteria of legumes, as had Janse previously in nodules of *C. muricata*. The latter referred to them as "bacterioden."

Meihe (8) published in 1918 the results of histological studies of nodules of *C. equisetifolia*, from Java, and ascribed their cause to a mycorrhizal fungus. To avoid confusion with the nodules of legumes he suggested use of the term "rhizothamnium" instead of "nodule."

In 1918, Narasimhan (9) read a paper before the Indian Science Congress at Lahore in which he stated that nodules were found on the roots of four *Casuarina* species, *equisetifolia*, *glauca*, *quadrivalvis*, and *stricta*, in Bangalore. He isolated a bacterium from nodules, presumably from one of these species, and liquid culture solutions, inoculated with this bacteria, showed an increase in nitrogen content after an incubation period of 35 days.

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[1] The writer wishes to acknowledge the aid given by Dr. W. R. Carroll, of the Department of botany and bacteriology of the University of Florida, under whose supervision the investigations were carried out; and to express his appreciation to the Division of Foreign Plant Introduction, U. S. Department of Agriculture, Washington, for the generous coöperation extended in furnishing seeds of numerous species.

McLuckie (7) presented a paper in 1923 before the Linnaean Society of New South Wales which included an account of thorough and detailed histological studies of nodules of *C. Cunninghamiana* as well as a description of bacteria isolated from them. Liquid cultures of the bacteria, like those of Narasimhan's, showed increases of nitrogen after incubation. He found infection threads, containing rod-shaped bacteria, within the cells and passing from cell to cell. The fungal hyphae referred to by Meihe may be the same as these threads. The bacteria are described as becoming transformed into bacteroids and as being similar to those causing nodules in leguminous plants. This author expresses the possibility of this organism being a species of *Pseudomonas*.

Reporting from India in 1924, Rao (11) gave data obtained from nodule cultures of an unnamed *Casuarina* species in which a progressive nitrogen increase occurred with the lengthening of the duration of the incubation period. His bacteria were rod-shaped with curved forms in old cultures.

Chaudhuri (3), in 1931, published results of his work with *C. equisetifolia* in which he isolated bacteria from nodules of trees growing in Madras. Negative results followed attempts to inoculate roots of that species with bacteria from 3 species of legumes, although he succeeded in inoculating 4 out of 20 *equisetifolia* plants with crushed nodules from the same host.

Parker (10), in 1932, reported the presence of root nodules on *C. equisetifolia*, *glauca*, and *montana* in the Punjab and United Provinces. He presented the assumption that root nodules are required for a satisfactory growth of *Casuarina* in certain soils; that one strian of the causal organism infects all species of the genus; and that the organism is not one of the ordinary strain of bacteria producing root nodules in legumes.

L. R. Toy, with the Alison Armour expedition in 1932, in correspondence with the author advised that he found root nodules on *C. triangularis* in Dominica and on an unidentified *Casuarina* species in Anguilla, B. W. I. A. F. Camp, of the Florida Agricultural Experiment Station, informed the writer in 1932 that he had observed nodules on the roots of a *Casuarina* species growing in Honduras.

None of the aforementioned investigators has reported the successful inoculation, as postulated by Koch, of any species of *Casuarina* with pure cultures of the organisms isolated and described, and none has presented data showing that nitrogen fixation occurs within the host or that a symbiosis obtains in the relation between the causal organism and its host.

THE HOST AND ROOT NODULES

The family *Casuarinaceae* consists of but a single genus, *Casuarina*, of which there are numerous species. The plants of the genus are leafless, evergreen trees or shrubs with wiry, erect or pendulous branches and numerous deciduous, verticillate, assimilatory branchlets. Because of the strong resemblance of the branchlets to the needle-like foliage of pines they are commonly considered as the leaves. The leaves, however, are replaced by whorls of minute scales which are inserted at the nodes, usually united at the base, and vary in number from 4 to 16 in the different species. The flowers are monoecious with the pistillate ones borne in axillary conelike spikes and the staminate ones terminally. The fruit is like a cone, varying in size and shape with the species, and contains many laterally compressed, winged nutlets.

The genus is indigenous to Australia, tropical Asia, the East Indies, and the South Pacific Islands and now has a wide tropical distribution in both hemispheres. Of the several species, some are now cultivated in the West Indies, Florida, and California in the United States, Mexico (15), Central America, Panama Canal Zone (16), Hawaii, and Uruguay (12).

In the lower peninsular portion of Florida the *Casuarinas*, there commonly called "Australian Pine," are planted extensively and in some places are becoming naturalized. Several species are grown, but the ones in greatest number probably are *C. equisetifolia, Cunninghamiana, and lepidophloia.* They are adapted to perhaps the widest range of uses of any exotic tree in the state, being utilized for windbreaks, closely clipped hedges, trimmed and untrimmed specimens, and for street and highway planting. The trees thrive on both acid and alkaline soils and are making a vigorous growth on dune sands, calcareous rocky soils, the muck of the Everglades, and the several sandy soil types of the lower peninsula. The species *equisetifolia* seems to withstand brackish soils and salt spray with no apparent injury and is planted extensively on the seacoast.

The nodules of *Casuarina* trees (pl. 1, fig. 1) differ materially from those of legumes in structure in that they are distinctly coralloid in form and have their beginning as a modified lateral root. In most instances a small rootlet protrudes from each nodule tip. Continued growth of the nodule, by branching and rebranching, results in compact masses of nodular tissue of varying sizes. Nodule masses with a diameter of 2½ inches have been noted. On small plants infection of the root system may be quite general or may be restricted to a single large clump of nodular tissue. Usually, however, infection in young plants is confined to a limited area of the root system in close proximity to the primary root. Histological and isolation studies have indicated that the causal organism apparently is a bacterium.

Field observations have been made of *Casuarina* trees in 20 widely separated places of the Florida peninsula and in no instance were trees above 6 feet high found without nodules on their roots. Trees of the genus are found on most soil types of that area and the soils on which those examined were growing had a reaction ranging from pH 5.20 to pH 7.98.

EXPERIMENTAL METHODS AND DATA

Nitrogen fixation

The method followed in the effort to determine whether fixation of nitrogen occurs in the nodule consisted essentially in growing in sand culture three groups of plants, of the same age and at the initiation of the test as nearly as possible of the same size, under uniform conditions except for variation in nitrogen supply. Plants of two of the groups were uninoculated, that is, they were without root nodules. One group of the two was given a nitrogen-containing (complete) nutrient solution at regular intervals and the other was supplied with a nutrient complete except for the omission of nitrogen. Plants of the third group were inoculated, i.e., they had nodules on their roots, and were given like amounts of the nitrogen-free nutrient as the one group of uninoculated plants. This grouping was as follows:

Group A. Inoculated plants (nodules on roots), given nutrient solution complete except for lack of nitrogen.

Group B. Uninoculated plants (without nodules on roots), given same nutrient solution as group A.

Group C. Uninoculated plants (without nodules on roots), given complete nutrient solution containing nitrogen.

The foregoing arrangement was based on the premise that if, under the carefully controlled conditions, the uninoculated plants supplied with the nitrogen-carrying nutrient (group C) showed evidence of greater thrift and measurable increases in growth over like plants given only the nitrogen-free nutrient (group B) the cause of the difference would definitely be due to the presence and availability of the element nitrogen. Further, if plants with nodules on their roots and whose sole supply of nutrient materials was from a nitrogen-free nutrient solution (group A) were to make a better growth than the nodule-free plants grown under identical conditions (group B), then the cause would be due to an element supplied by the organism of the nodules, and if the nodulated plants (group A) made a growth comparable to those of the group supplied with nitrogen (group C), then the element furnished through the nodules must be nitrogen. Thus it is apparent that, upon the fulfillment of such conditions, both the fixation of atmospheric nitrogen by the organism of the nodule and a symbiotic relationship between the organism and its host would be established.

On the basis suggested in the foregoing three series of like experiments were carried out, the first two with one species, *C. equisetifolia*, and the last with four species, *C. Cunninghamiana*, *equisetifolia*, *glauca*, and *lepidophloia*. Differences, resulting after a cultural period of several months, in appearance, growth increment, and nitrogen content of the plants of the different groups constitute the basis of the conclusions drawn. The four species are large trees in maturity, and their woody nature accounts for the apparently slow growth as compared to that of herbaceous plants and for the relatively long periods of time they were held in culture.

A preliminary set-up was made by dividing into two groups young plants, of the same age and from the same lot of seed, grown individually in soil in 4-inch porous flower pots. The seeds prior to planting were disinfected by immersing for 3 minutes in a 1:1,000 mercuric bichloride solution, and the soil was sterilized by heating twice in small containers in an autoclave at 15 pounds steam pressure for 3 hours, with a lapse of not less than 24 hours between sterilization periods. All pots or jars were sterilized in a solution of bichloride of mercury. Trees of one of the groups were inoculated by means of small nodule pieces which were inserted in the soil near the stem of the plant at a depth of about 1 inch. The nodules previously had been immersed for 3 minutes in a 1:1,000 mercuric bichloride solution and then thoroughly rinsed. When nodules became visible on the inoculated plants, which usually occurred after approximately 4 months, the nitrogen-fixation experiments were inaugurated.

Trees of as near the same size as possible from the two groups, one with root nodules and the other without, were then selected, their roots carefully washed free of all soil particles, and planted in like quantities of washed, coarse sand in 1-gallon glazed jars. The jars were fitted with a small hole at the bottom, plugged with glass wool, for drainage. The trees were then segregated into the groups A, B, and C, as previously outlined.

Plants of groups A and B were given a nutrient solution complete except for nitrogen (solution B) and those of group C were given a complete nutrient solution containing nitrogen (solution A). The nutrient solutions used were made up in distilled water and were the same as those of Ginsberg (4) which were based on those of Shive (14). Their composition is given in table 1. The sand for each lot was moistened with like quantities (1 liter) of the respective nutrient solution at the time of planting and the jars, including sand and tree, were weighed daily and kept to constant weight by the addition of distilled water. At 10-day intervals in series I and II and at weekly intervals in series III, 150 cc. of the designated solution for each group was given each of the plants of that group. All plants for the duration of the experiment received identical amounts of their respective nutrient solutions.

TABLE 1

Composition of nutrient solutions

SOLUTION	$Ca(NO_3)_2$ PER LITER	$MgSO_4$ PER LITER	KH_2PO_4 PER LITER	$Ca(H_2PO_4)_2$ PER LITER	K_2SO_4 PER LITER
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
A	0.8533	1.8060	2.4507	......	......
B	......	1.8060	......	2.4507	0.8533

A few drops of a weak solution of $FeSO_4$ were given the plants at bi-weekly intervals.

Series I. The plants, *C. equisetifolia,* of this series were approximately 12 months old when transferred to the sand culture. Those with nodules had been inoculated at an age of about 6 months, but no nodules were noted on their roots until about 5½ months later. At the time of their removal from soil and placing in sand there was but little difference in the size of the plants but the color of the foliage[2] of those with nodules was a somewhat deeper green than that of the uninoculated plants. Eighteen plants constituted the series, six in each of the groups A, B, and C, as outlined. Three plants from each group were removed at the expiration of 142 days and analyzed for total nitrogen. In all nitrogen determinations the Gunning modification of the Kjeldahl method was used, the material previously having been dried at 70°C. for not less than 24 hours and then finely ground in a Wiley mill.

Table 2 shows quite definitely the wide difference in growth that occurred during the 142-day experimental period between the inoculated plants (group A) and the uninoculated plants (group B) receiving the same nutrient solution

[2] The term "foliage" as used in this paper has reference throughout to the assimilatory branchlets.

containing no nitrogen (solution B). In contrast was the comparable growth of the inoculated plants of group A which were given no nitrogen (solution B) and those of the uninoculated group C which received a nitrogen-containing nutrient (solution A). The same marked trend is also readily apparent in the total nitrogen content of the plants of the three groups. All trees of groups A and C were thrifty at the end of the experimental period and had a thick foliage that was of a deep green color, whereas all of the trees of group B had a sparse, yellowed foliage and a very unhealthy appearance. The general appearance of plants of series I at the initiation and conclusion of the experiment is shown in plate 1, figures 2 and 3, and of typical root systems at the end of the test in plate 2, figure 1.

TABLE 2

Series I—Casuarina equisetifolia

Placed in sand culture 10–7–31; removed from culture 2–26–32 (142 days)

GROUP	PLANT NUMBER	TREATMENT	NUTRIENT SOLUTION*	HEIGHT		HEIGHT INCREMENT		WEIGHT WHOLE PLANT AT REMOVAL (OVEN-DRY)	PER CENT NITROGEN OVEN-DRY BASIS WHOLE PLANT
				At start	At removal				
				cm.	*cm.*	*cm.*	*per cent*	*gm.*	
A	6	With nodules	B	53.3	71.1	17.8	33.4	17.1	1.22
	20	With nodules	B	45.7	55.9	10.2	22.3	8.7	1.37
	31	With nodules	B	43.2	53.2	10.0	23.1	9.7	1.12
B	24	Without nodules	B	35.6	35.6	0.0	0.0	1.0	0.51
	33	Without nodules	B	38.1	39.4	1.3	3.4	1.9	0.60
	35	Without nodules	B	45.9	46.3	0.4	0.9	1.4	0.51
C	22	Without nodules	A	38.1	45.6	7.5	19.7	6.0	1.47
	27	Without nodules	A	45.5	58.4	12.9	28.4	8.7	1.57
	15	Without nodules	A	38.2	43.3	5.1	13.3	5.8	1.37

* See table 1.

Series II. Trees of this series were three of each group of series I that were carried on in culture for a period of 347 days. The differences in growth increment continued the same trend as those of series I but were much more pronounced with the increase in duration of the test. Comparative data are given in table 3. It will be noted that the increase in height in the plants of group A ranged from 38.1 cm. to 74.9 cm. (71.5 to 218.4 per cent); of group B from −5.0 cm. to 10.2 cm. (0 to 30.1 per cent); and of group C from 3.2 cm. to 25.6 cm. (7.4 to 59.6 per cent). The most significant difference noted between the trees of series I and II was in composition, the trees of group C of the latter series having a lower nitrogen content than those of group A—a reversal of the condition found in the two groups of series I at the time of analysis.

That those uninoculated trees of group C, supplied with a nitrogen-containing

TABLE 3

Series II—Casuarina equisetifolia

Placed in sand culture 10–7–31; removed 9–19–32 (347 days)

GROUP	PLANT NUMBER	TREATMENT	NUTRIENT SOLUTION*	HEIGHT		HEIGHT INCREMENT		STEM DIAMETER AT REMOVAL	WEIGHT (OVEN-DRY) AT REMOVAL			PER CENT NITROGEN (OVEN-DRY BASIS)			REMARKS
				At start	At removal				Foliage	Stem	Roots	Foliage	Stem	Roots	
				cm.	*cm.*	*cm.*	*per cent*	*cm.*	*gm.*	*gm.*	*gm.*				
A	1	With nodules	B	45.7	88.3	42.6	93.2	0.89	44.30	24.55	14.70	1.30	0.75	0.45	All of group vigorous
	28	With nodules	B	34.3	109.2	74.9	218.4	0.82	.....	.....	.....	....	....	....	Not removed from culture
	5	With nodules	B	53.3	91.4	38.1	71.5	0.82	.....	.....	.....	....	....	....	Not removed from culture
B	23	No nodules	B	33.0	43.2	10.2	30.1	0.40	5.50	1.85	2.90	0.71	0.71	0.38	Foliage yellow on all of group
	11	No nodules	B	40.6	35.6	−5.0	0.0	0.40	.....	.....	.....	....	....	....	Original top of stem died
	10	No nodules	B	35.6	43.5	7.9	22.2	0.33	.....	.....	.....	....	....	....	Not removed from culture
C	26	No nodules	A	48.3	73.1	24.8	51.3	0.71	11.10	9.20	5.65	1.10	0.75	0.46	All of group thrifty
	12	No nodules	A	48.2	51.4	3.2	7.4	0.64	.....	.....	.....	....	....	....	Top died back early in cultivation period. Not removed from culture
	18	No nodules	A	43.0	68.6	25.6	59.6	0.71	.....	.....	.....	....	....	....	Not removed from culture

* See table 1.

nutrient (solution A), did not attain the size of those with nodules of group A was due, quite probably, to the use of an insufficient supply of the nutrient to give the plants the amounts of nitrogen required for maximum growth. With the plants in group A, furnished no nitrogen whatever in the nutrient solution, there was every evidence indicating that their nitrogen requirements were amply taken care of by the only possible source of supply—the nodule-forming organisms of their roots.

Series III. Four species, *C. glauca*, *lepidophloia*, *Cunninghamiana*, and *equisetifolia*, were included in this series. All were approximately 10 months old at the time they were segregated and one of the groups of each species was inoculated. Nodules were found to be present on all of the inoculated plants on the 109th day after inoculation, and all plants were then transferred to the sand medium and grouped as in the previous series. Each group, A, B, and C, was composed of two trees of *equisetifolia* and one each of the other species. The *lepidophloia* plant in group C was lost early in the experiment through accidental dropping of the pot and spilling of the contents when weighing.

Measurements and analyses were made of the plants after they had been in culture 102 days. Full data are given in table 4, which shows that not only height, weight, and diameter of stem but also nitrogen content of the plants of both groups A and C increased far more than did those of group B and that average increases made by the nodulated plants of A were comparable with those made by the nitrogen-supplied trees of group C. The height increment in group A ranged from 14.0 cm. to 35.5 cm. (17.5 to 37.5 per cent); in group B from 0 cm. to 5.1 cm. (0 to 6.0 per cent); and in group C from 11.4 cm. to 35.6 cm. (13.8 to 49.1 per cent). The stem diameter increment in group A ranged from 0.32 cm. to 0.50 cm. (58.2 to 90.9 per cent); in group B from 0.02 cm. to 0.10 cm. (0.4 to 1.8 per cent); and in group C from 0.27 cm. to 0.53 cm. (45.0 to 100.0 per cent). The differences in oven-dry weight of the plants at removal and the differences in percentages of nitrogen contained show that the total amounts, by weight, of nitrogen in the plants of groups A or C exceeded by many times the amounts in the plants of group B.

It was further evidenced, from comparison of the plants of groups B and C, that lack of nitrogen was the limiting growth factor and that adequate nitrogen for vigorous growth was furnished the plants of group A through their nodules. In all instances, with each of the four species, the plants of group A made a vigorous growth and were of excellent appearance while all plants of group B became yellow and sparsely foliaged and assumed a very unthrifty appearance. Both *equisetifolia* plants of group B were without enough foliage for analysis at the completion of the test period. Figures 2 and 3 of plate 2 show plants of this series at the beginning and end of the 102-day period in culture.

An analysis of nodules, from plants grown in soil, showed them to have a nitrogen content of 1.5 per cent on an oven-dry basis. This was considerably above the percentage contained in roots of nodulated plants but did not greatly exceed that of the whole plant.

TABLE 4

Series III—Casuarina, equisetifolia, Cunninghamiana, lepidophloia, *and* glauca

Placed in sand culture 8–16–32; removed 12–27–32 (102 days)

GROUP	PLANT NUMBER	SPECIES	TREATMENT	NUTRIENT SOLUTION*	HEIGHT		HEIGHT INCREMENT		STEM DIAMETER		STEM DIAMETER INCREMENT		WEIGHT (OVEN-DRY) AT REMOVAL WHOLE PLANT	PER CENT NITROGEN (OVEN-DRY BASIS)			REMARKS
					At start	At removal			At start	At removal				Foliage	Stem	Roots	
					cm.	*cm.*	*cm.*	*per cent*	*cm.*	*cm.*	*cm.*	*per cent*	*gm.*				
A	34	equisetifolia	With nodules	B	81.3	111.8	30.5	37.5	0.62	1.10	0.48	77.4	....	....	....	....	Not removed from culture.
	7	equisetifolia	With nodules	B	85.1	107.3	22.2	26.1	0.55	1.05	0.50	90.9	81.7	1.45	0.73	0.83	Vigorous
	14	Cunninghamiana	With nodules	B	82.6	108.0	25.4	30.8	0.60	0.95	0.35	58.3	46.4	1.86	0.62	1.00	Vigorous, well-branched
	21	lepidophloia	With nodules	B	80.0	94.0	14.0	17.5	0.55	0.87	0.32	58 2	28.9	1.59	0.65	0.92	Vigorous
	25	glauca	With nodules	B	85.1	101.6	16.5	19.4	0.56	0.96	0.40	71.4	45.5	1.32	0.58	0.91	Vigorous
B	26	glauca	No nodules	B	66.7	69.9	3.2	4.8	0.45	0.47	0.02	0.4	17.4	0.58	0.26	0.63	Foliage thin and yellow
	22	Cunninghamiana	No nodules	B	85.1	90.2	5.1	6.0	0.54	0.62	0.08	1.5	20.0	0.57	0.32	0.63	Foliage sparse and very yellow
	4	equisetifolia	No nodules	B	58.4	61.0	2.6	4.5	0.45	0.49	0.04	0.8	....	....	....	....	Not removed from culture. No foliage. Plant nearly dead
	27	lepidophloia	No nodules	B	61.0	61.0	0.0	0.0	0.56	0.60	0.04	0.7	22.4	0.40	0.30	0.55	Foliage very sparse and very yellow
	2	equisetifolia	No nodules	B	82.6	83.8	1.2	1.5	0.55	0.65	0.10	1.8	11.4	....	0.29	0.61	No foliage, plant almost dead
C	24	equisetifolia	No nodules	A	55.9	73.7	17.8	31.8	0.43	0.86	0.43	100.0	....	....	....	....	Not removed from culture. Vigorous
	39	equisetifolia	No nodules	A	82.6	94.0	11.4	13.8	0.60	0.87	0.27	45.0	42.4	1.01	0.40	0.69	Vigorous
	15	Cunninghamiana	No nodules	A	72.4	108.0	35.6	49.1	0.55	1.08	0.53	96.4	39.7	0.98	0.37	0.84	Vigorous
	33	glauca	No nodules	A	75.6	94.0	18.4	24.3	0.57	0.94	0.37	64.9	34.3	0.91	0.57	0.89	Vigorous

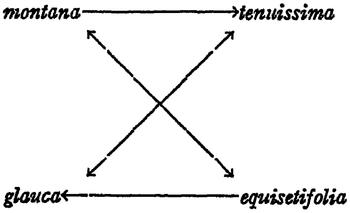
Cross-inoculation of species

The nodules for the investigations were obtained from Homestead, Florida, from roots of *C. lepidophloia* and, unless contamination has occurred from an unsuspected source, which is believed highly improbable, all inoculations herein reported had their origin in this single lot of nodules. It was found during the late autumn of 1930 that inoculation was readily accomplished by the insertion of one or two small pieces of a fresh nodule about the roots of an uninfected plant. No special technique was required to secure infection; a small hole from ½ to 1 inch in depth was made in the soil at the base of the plant and the nodule piece dropped in and covered. No effort was made to insure contact of the nodule piece with roots of the plant. Prior to use for inoculation purposes the nodules were immersed in a disinfectant solution as previously described. It was easily possible by this method to secure infection of 90 per cent of the plants so treated.

The time required for infection and appearance of nodules on the roots apparently varies with the age or size of the tree. Small plants may require 5 to 6 months before nodules are seen whereas those plants with a stem diameter of about ¼ inch have shown nodules on the 70th day after inoculation.

All plants subjected to inoculation were grown from disinfected seed in sterilized soil and sterilized pots, and when comparative tests were made control plants were provided. The plants were grown in a greenhouse with the pots suspended in racks over deep pans of oiled water to prevent contamination by crawling insects. That the sterilization methods were effective was shown by a group of several plants that was held for over 2 years without trace of infection.

Inoculation with nodule pieces, as indicated in the foregoing, has resulted in infection with typical nodule formation on the roots of the nine following *Casuarina* species: *Cunninghamiana, lepidophloia, equisetifolia, montana, Fraseriana, sumatriana, glauca, tenuissima, triangularis.* Successful cross-inoculations, by nodule pieces, have been made with the following species in the following manner:



DISCUSSION AND CONCLUSIONS

Results from the three series of nitrogen-fixation experiments were remarkably uniform and conclusive and in no instance was there a plant of any of the four species in any group that did not follow the general reaction of the other plants of its group. Throughout, the uninoculated plants that were supplied with a nitrogen-free nutrient showed a decline with an entire lack of thrift, a yellowing and loss of foliage, no appreciable growth, and a low nitrogen content. Like plants that were furnished nitrogen in a complete nutrient continued in every instance in vigorous growth, carried a heavy, deep green foliage, and in composition contained a much greater percentage of nitrogen. Those plants with nodules on their roots and which were furnished only a nitrogen-free nutrient were without exception comparable with, or above the average of, the nitrogen-supplied, uninoculated plants in growth increment and vigor, appearance, and percentage of nitrogen contained. Growth differences became correspondingly marked with increases in duration of the cultural period.

Inoculation experiments which were carried out prove beyond question that the nodules are due to the presence of an organism, and investigations of others, as well as the writer, indicate the causal organism to be a bacterium. The results also have shown that inoculation by means of nodule pieces can be successfully accomplished and that nine *Casuarina* species were susceptible to infection with resultant nodule formation on the roots. Since the nodules for inoculation had their origin in a single lot taken from one species and from them nodule formation was obtained in eight other species it is considered that a single strain of the causal organism is capable of infecting those nine species and possibly, as well, all species of the genus subject to infection.

Based on the very definite reaction in appearance and thrift, growth increment, and nitrogen percentages contained in inoculated and uninoculated (or infected and non-infected) plants grown in a sand medium under controlled conditions as to supply and kinds of nutrient materials available there can be no doubt, in the association existing between the organism of the *Casuarina* root nodule and its host, that fixation of atmospheric nitrogen occurs in appreciable amounts and that the relation is truly symbiotic.

Observations made of *C. equisetifolia* in Florida show the trees to be able to maintain a thrifty growth on the dune sands near the coast where few other than the truly littoral plants are able to exist unless supplied with nitrogenous fertilizers. The trees of this and other species are also grown in the inland sections where the soils are of greater natural fertility and there again the trees thrive without additions of nitrogen to the soil. Two or three species are utilized to some extent as windbreaks for citrus and other fruit plantings. In several instances it has been noted that the thrift of a row of citrus trees immediately adjacent to a closely planted *Casuarina* windbreak apparently was not impaired by the close association, other than for slight mechanical injury from intermingling of branches or from too dense shading. In those instances the encroachment of *Casuarina* roots in the soil area of the citrus roots seemingly had no ill effect on the citrus trees, since the latter were in every respect, as far as could be noted, comparable with like trees 150 feet distant even though they were furnished no additional fertilizers to compensate and the *Casuarina* trees had none except that taken from the citrus row. Symbiotic nitrogen fixation in the *Casuarinas* offers the explanation for these conditions.

Granting the supposition that little nitrogen may actually be released in the soil for the benefit of other plants, which is not yet known, the information that the tree is capable of supplying its own nitrogen requirements is of importance and value in the choice of trees for both windbreak and other plantings.

SUMMARY

Trees of the genus *Casuarina* are among the non-leguminous plants having nodules on their roots. The native habitat of the several species of the genus is in the tropical portions of Asia, Australia, and the East Indies, but many species now have widespread distribution in the tropics and subtropics of the western hemisphere, including the warmer parts of the Florida peninsula. The trees can maintain a vigorous growth in dune sands and other soils of low fertility without application of nitrogenous fertilizers.

Of numerous *Casuarina* trees examined in 20 different locations in Florida, none above 6 feet in height was found without nodules on its roots.

Morphologically the nodules of the *Casuarina* species differ materially from those of the legumes in that they are modified lateral roots and develop by successive branching into compact coralloid masses that may attain a diameter of over 2 inches. The causal organism apparently is a bacterium.

Inoculation of non-infected plants growing in pots in sterile soil was accomplished by inserting small pieces of fresh nodules in the soil about their roots. From 70 days to 5½ months were required for appearance of nodules on the roots after inoculation.

Nine *Casuarina* species, *Cunninghamiana*, *equisetifolia*, *Fraseriana*, *glauca*, *lepidophloia*, *montana*, *sumatriana*, *tenuissima*, and *triangularis*, were found to be susceptible to infection, nodules having been produced on their roots by inoculation with nodule pieces.

Cross-inoculations, by nodule pieces, indicate that a single strain of the causal organism is capable of infecting the nine species.

The comparative growth reactions of four species, *C. Cunninghamiana equisetifolia*, *glauca*, and *lepidophloia*, with and without nodules, grown under controlled conditions as to nitrogen supply in a sand medium, have established that fixation of atmospheric nitrogen occurs in the nodules and that the relation of the host and causal organism of the nodule is one of mutualistic symbiosis.

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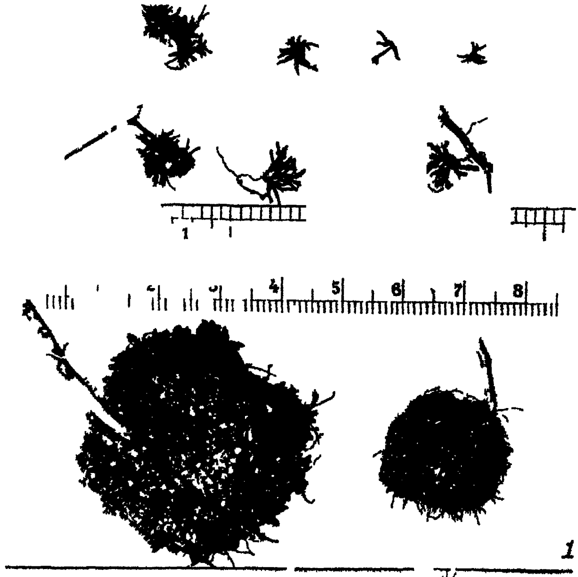
PLATE 1

FIG. 1. Root nodules of *Casuarina.*

FIG. 2. Series I. *C. equisetifolia.* Typical appearance of the plants of the three groups at beginning of the experiment.

FIG. 3. Series I. *C. equisetifolia.* Typical plants of the three groups after 142 days in culture. Left to right

No. 18 from Group C—without nodules, nitrogen supplied in nutrient.
No. 11 from Group B—without nodules, no nitrogen in nutrient.
No. 31 from Group A—with nodules, no nitrogen in nutrient.



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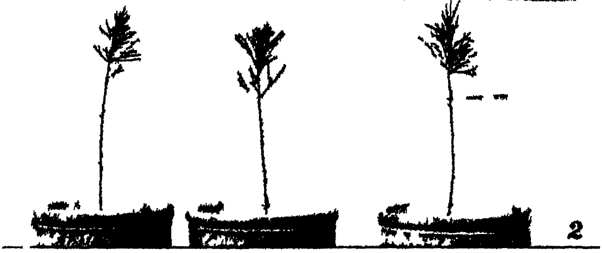

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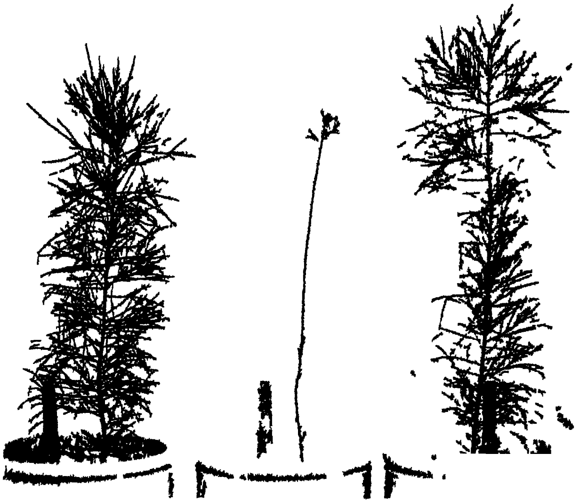
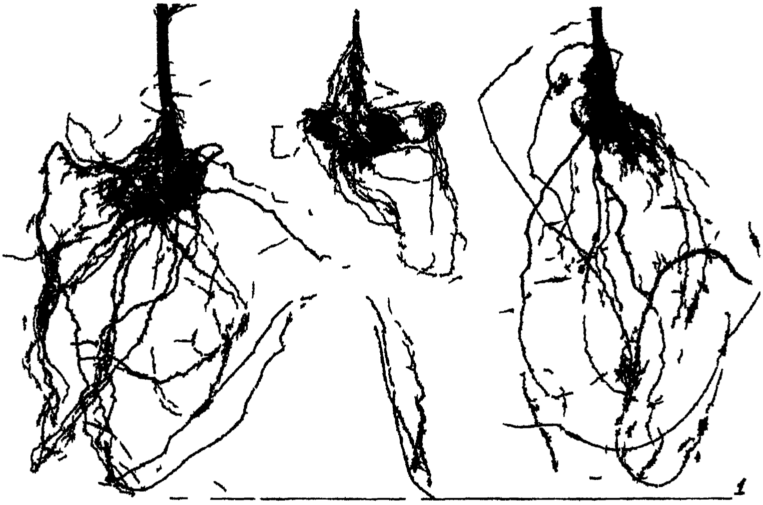


PLATE 2

Fig. 1. Series I. *C. equisetifolia.* Root systems of the three groups after 142 days in culture. Groups A, B, and C from left to right. Nodules can be noted at base of stem on plant of Group A (left). The apparent enlargements on roots of plant on right, from Group C, are not nodules but brushlike collections of very fine rootlets.

Fig. 2. Series III. *C. equisetifolia.* Plants at time of placing in culture.
Nos. 34 and 7—Group A.
Nos. 2 and 4—Group B.
Nos. 39 and 24—Group C.

Fig. 3. Series III. *C. equisetifolia.* Appearance of plants after 102 days in culture.
Nos. 34 and 7, Group A—With nodules, no nitrogen in nutrient.
Nos. 2 and 4, Group B—without nodules, no nitrogen in nutrient.
Nos. 39 and 24, Group C—without nodules, nitrogen supplied in nutrient.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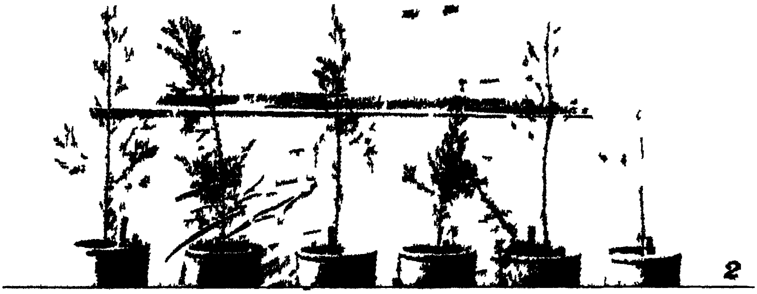
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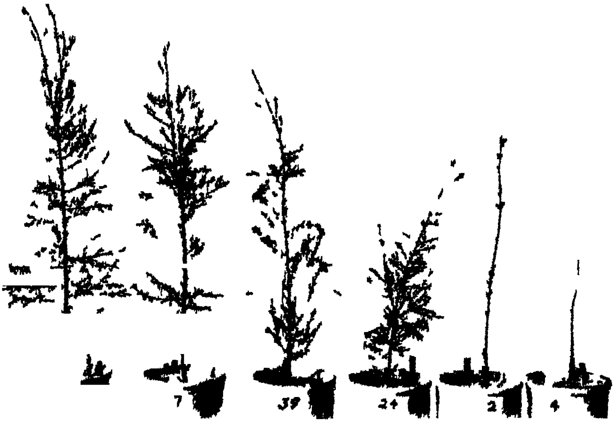

A STUDY OF SOME SALT WATER FLOODED SOILS ON THE EASTERN SHORE OF VIRGINIA

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During a rather severe storm in March, 1932, several thousand acres of farm land on the bayside area of Accomac County on the Eastern Shore of Virginia were flooded with salty water from the Chesapeake Bay. The sea water covered the fields to a depth of several feet in some instances and remained upon the soil from a few hours to several days. The length of time that the water remained upon the soils was governed principally by the soil types and systems of drainage in the different fields. In the poorly drained, heavy soil types the water remained upon the ground much longer than it did upon the more sandy places. Some fields were surrounded by a system of dikes, and in these instances the banks were either washed away or opened, which allowed the salt water to recede from the soil very rapidly.

The salt content of the water that covered the soils may have varied somewhat since there were some sources of fresh water running into the Bay. The extent that the content of salt in the water varies is an indefinite factor and no doubt influenced the salt absorbed by the different soils. In 21 determinations of the saline content of the Atlantic Ocean, Hilgard (3) states that the minimum and maximum variation was 3.506 to 3.710 per cent. Common salt constitutes from 75 to 80 per cent of the mineral residue. Magnesium salts also make up a high percentage of the rest of the mineral residue. The calcium content is noticeably low.

The entire area covered by the sea water is only a few feet above sea level. In spite of the fact that the ground is very close to sea level, the tidal water covers the soil only on exceptional occasions. The March storm is reported to have been the worst within the memory of some of the oldest inhabitants. A similar, but much smaller, flooding of the soils was reported to have occurred some 20 or 30 years ago. The severity of that storm could not be learned. The injury caused by any flooding of the soil with salty water depends a great deal upon the weather conditions that prevail after the flooding occurs.

These lands were flooded on March 6, 1932, after a severe rainy period. The soil was thoroughly wetted when the sea water covered the ground. The storm ceased, however, immediately after the salt water covered the soil and no further rain fell for some weeks. The summer that followed this storm was abnormally dry and the rainfall rather evenly distributed, which aggravated the existing condition. The cropped soils that were flooded were used principally for the production of strawberries, potatoes, lima beans, string beans, and onions.

In order to trace the movement of the salt in the soil, its effect upon the soil, and upon plant growth, the following research was attempted.

SALT CONTENT AND SOIL CONDITION

In general the predominating soil types in the flooded area are the well-drained sands and sandy loams. In some instances the soils are sandy underlain by clays, and some of the lower lying soils are fairly heavy sedimentary soils.

The chemical analysis of the first series of soil samples taken from the flooded area showed that the sodium chloride content of the soil was influenced not so much by the length of time that the salt water stood upon the land as by the physical condition of the soil itself. The texture and soil tilth influenced the salt absorbing power of the soil more than did any other factors observed. The porous soils absorbed more salt than did the compact ones and the freshly cultivated soils took up several times as much salt as did those that had been uncultivated or wintered over in grain. Table 1 serves to bring out these points.

TABLE 1

Salt content of the cultivated horizon of the cropped soils in relation to soil tilth

SOIL TILTH	NaCl	STANDARD DEVIATION
	per cent	
Plowed immediately before flooding: Average of nine fields....	0.315	0.034
Plowed some time before flooding: Average of eight fields.....	0.131	0.033
In grain crop, not plowed: Average of five fields............	0.073	0.016
Not flooded: Average of five fields.........................	0.052	0.016
Subsoil in flooded area: Average of three fields...............	0.058	0.011
Subsoil in unflooded area: Composite from one field..........	0.010	.....

The light, open sands and the soils freshly plowed absorbed more salt from the sea water because of the open texture of the soil. In the porous soils the sea water passed through the soil whereas in the compact ones it ran off. Heavy rains immediately before the flooding filled the low lying soils with fresh water, which prevented them from absorbing as much salt as possibly would have been absorbed otherwise. The salt water ran off the compact soil as soon as the tide subsided, whereas the freshly plowed soils held much of the water even after the tide had receded. In some cases, however, where the tidal water remained upon the land for a long time the heavier types of soil took up considerable salt.

INFLUENCE OF THE SALT WATER FLOODING OF THE SOIL UPON THE REPLACEABLE BASES OF THE SOIL

Inasmuch as the surface soil was in contact with the salt water, for a rather long while in many instances, the cations of the salts of the sea water would be expected to replace some of the cations in the soil. In table 2 one notes that the replaceable calcium has been removed but the magnesium and potassium have not. As a matter of fact, the sea water has effected an increase in mag-

TABLE 2

The effect of sea water flooding of sandy soils upon the replaceable cations in the surface 0–3½ inch soil horizon

Soils flooded March 6; sampled March 20

ORDER	APPROXIMATE TIME FLOODED	CONDITION OF SOIL AT TIME OF FLOODING	M.E. PER 100 GM. OF SOIL				pH NATURAL SOIL	pH* AFTER LEACHING
			CaO	MgO	K_2O	Na_2O		
		Field No. 1						
1	18 hours	Plowed fairly fresh before storm	0.35	0.77	0.43	1.24	4.7	5.9
2	12 hours		0.42	0.40	0.11	0.74	5.1	5.6
3	Not flooded†		0.82	0.21	0.19	0.38	5.3	5.1
		Field No. 2						
1	18 hours	Plowed some time before storm	1.29	0.69	0.28	1.92	4.8	6.2
2	Not flooded†		1.77	0.37	0.25	0.55	5.2	5.9
		Field No. 3						
1	6–8 hours	Plowed in fall	1.18	0.74	0.25	1.95	5.5	5.8
2	6–8 hours	Plowed fresh before storm	0.97	0.88	0.29	3.64	4.9	6.2
		Field No. 4						
1	Not flooded†	Plowed before storm	1.77	0.37	0.22	0.55	5.2	5.6
2	6–8 hours		1.45	1.05	0.26	1.39	5.0	5.8
		Field No. 5						
1	6 hours	Plowed in fall	1.33	0.85	0.19	1.29	4.7	5.9
2	12 hours		0.78	0.99	0.17	2.08	4.9	5.9
		Field No. 6						
1	6–8 hours	Plowed in summer before storm	1.77	1.05	0.09	1.66	5.0	5.8
2	6–8 hours	Plowed after storm	2.34	0.76	0.09	0.81	5.5	5.6

* Easily soluble salts removed; pH by quinhydrone electrode.

† Possibly subjected to spray of salt water.

nesium and a slight increase in replaceable potassium. This is in accord with the work of Page and Williams (8) and with that of Hissink (4). Again, Russell (9) discusses the work of T. S. Dymond of Essex and others in which a similar relation is observed.

Magnesium salts make up a considerable portion of the saline content of the sea water, and potassium salts are also present. In the light of base exchange work magnesium and potassium would be expected to enter the soil colloid at the expense of the calcium ions. The mass effect of the high sodium chloride content of the sea water would replace much of the replaceable calcium and hydrogen. We observe that the pH of the soils with high sodium chloride content is lower than that of the ones with lower salt content, but after the water-soluble ions of the soil have been leached out the reverse is true. This indicates that the sodium and magnesium ions of the sea water have entered the soil colloid complex to a greater extent than the replacement of the other bases, thereby giving a colloid more completely saturated with bases.

SOIL SALT CONTENT AND CLIMATIC CONDITIONS

The preceding sections deal with some of the effects that the sea water had upon the soil as it passed over the land. Now we shall deal with the movement of the salt that remained in the soil after the flood waters were removed. The salt content of the open sandy soils would be expected to be leached from the soil very rapidly. This no doubt would occur if there were abundant rainfall following the flooding. Following the flooding of the lands on the Eastern Shore, however, a rather dry summer was experienced. The tendency under these conditions was for the salt to accumulate on top of the ground during dry periods and be washed down into the soil during wet periods. Table 3 shows the way the salt content of the surface 0–3½-inch horizon varied in relation to the rainfall.

TABLE 3

Average salt content in surface soil and rainfall*

	MARCH 20	APRIL 13	MAY 10	MAY 30	JULY 13	DEC. 12
Per cent NaCl	0.150	0.103	0.195	0.256	0 341	0.063
Inches† rainfall between dates		3.73	1.68	1 30	0.68	18.62

* Average results from eight different fields, 0–3½ inch horizon.

† Rainfall data obtained from Onley, Va., several miles away from the area flooded with salt water.

There would be expected much less leaching in the summer months due to increased evaporation than during winter months. This factor would prevent the leaching of salt from the soil during summer months, particularly during light rainfall.

In a study of the salt content in the different soil horizons, in the soil samples taken in December it is noted in table 4 that the salt has been fairly well leached down into the soil. The total salt content of the soil to a depth of 40 inches was less in the December sampling than found in the first 9 inches of soil on May 30. On this basis, then, the sum total of the salt in the soil to the 40-inch depth would hardly produce plant injury, inasmuch as it would not again be concentrated in the surface 0–3½-inch horizon by evaporation.

TABLE 4

Salt content in different soil horizons

ORDER	DEPTH	NaCl* MAY 30	NaCl* DEC. 12
	inches	*per cent*	*per cent*
1	0– 3	0.287	0.047
2	3– 6	0.065	0.047
3	6– 9	0.041	0.022
4	9–12	.....	0.023
5	12–18	.....	0.028
6	18–24	.....	0.024
7	24–36	.....	0.018
8	36–40	.....	0.010
Total pounds per acre..................		3,930	3,250

* Average from three fields.

SALT CONTENT AND PLANT INJURY

Page and Williams (8) and Hissink (4) in a study of this nature with some heavy clay soils found that the principal injury to crops was due to a bad physical condition developed from the sodium clay formed by the action of the sea water. Page and Williams also quote a passage from a publication by Prof. J. M. van Bemmelen in which he stated that the chief trouble with some heavy sea water flooded clays that he studied was the bad physical conditions developed after the salt had been somewhat washed out of the soil. The soils herein examined were much lighter and more open than the soils previously studied. The most of our soils were too sandy for the formation of a bad physical condition. Our serious factor in plant growth was the actual accumulation of toxic concentration of sodium chloride in the surface soil.

The situation herein developed did not vary greatly from some of the arid regions of the United States where the soil is likely to contain an accumulation of soluble salts in such quantities that the growth of certain vegetation is hindered. The chief difference in this situation is in the kinds of salts. Sodium chloride was the chief accumulated salt in our soils whereas sodium sulfate and carbonate as well as the chloride make up the principal part of many of the alkaline soils of the arid regions. The alkaline soils of the arid portions of the United States have been investigated rather thoroughly in regard to salt injury to vegetation by Hilgard (3), Harris (2), Dorsey (1), and others (6, 7). In consideration of the physical, chemical, and biological factors of the reclamation and treatment of the alkaline soils and likewise their effects upon plant growth, attention should be called to the work of Joffe and McLean (5) in which a review of the literature is reported with special attention to the publications of K. K. Gedroiz on this subject. The work of these investigators has brought out certain salient factors which warrant consideration here. The anions appear to be more toxic to plants than the cations. Chlorine is the most toxic anion and sodium is the most toxic cation found in the alkaline soils. These two ions, then, are the ones that concern us most in this type of work, for they are the predominating ions present in sea water. The logical conclusion that it takes less salt to produce injury in sands than in the heavy clays has been brought out, but on the other hand the injury to the physical condition of the soil is far greater and longer lasting in the clays. It is further shown that the percentages of soil moisture, nature of the ions, proportion in which they appear, etc., influence the injury to plants. It is pointed out also that recoverable chlorides to the extent of 0.2 per cent or over are likely to produce injury to ordinary crops in coarse sands.

TABLE 5

Relation of soil organic matter, salt content, pH value, and plant injury

May 10, 1932

ORDER	CONDITION OF PLANT	pH	NaCl	LOSS ON IGNITION
			per cent	*per cent*
1	Not injured	6.5	0.097	4.28
2	Not injured	5.6	0.108	3.30
3	Not injured	5.1	0.134	3.38
4	Not injured	5.8	0.153	3.42
5	Not injured	4.7	0.163	3.56
6	Injured	4.3	0.238	3.50
7	Injured	5.0	0.374	8.24
8	Injured	4.8	0.255	5.71
9	Injured	5.0	0.458	7.98
10	Dead	4.6	0.231	3.09
11	Dead	4.7	0.369	3.85
12	Dead	4.7	0.368	5.03
13	Dead	4.1	0.745	6.75

In a series of germination studies with several of the vegetables in the presence of increasing quantities of sodium chloride we found 0.2 per cent salt to be serious in the light sandy soils low in organic matter. Not only was germination delayed, but also the young plants showed distinct signs of injury. The addition of calcium as nitrate or as lime somewhat offset the toxic conditions. Field tests substantiated the foregoing results. Lime under field conditions facilitated the leaching of the salt and stimulated plant growth. Fertilizers prepared from chlorine-free salts proved beneficial in trial tests on soils analyzing 0.185 per cent water-soluble chlorine. Again under field conditions we found that plants growing on soils with high organic matter withstood more salt than the soils with low organic matter content. The information collected was for the strawberry plant. In general, this same relation held true for other crops but since strawberries is the principal crop of this section, we were able to obtain more systematic data upon it. In table 5 are given the data in regard to the injury to strawberry plants. These data are by no means complete, but they offer some basis for conclusions. For example, a soil with about 8.0 per cent loss on ignition and about 0.45 per cent salt showed only injury to strawberry plants, whereas a soil with 3.0 per cent loss on ignition and 0.23 per cent salt killed the plants outright.

METHODS USED

The chlorine was estimated in a 1:4 soil:water extract and the chlorine titrated with silver nitrate using potassium dichromate as the indicator. From the chlorine content the sodium chloride was calculated. The replaceable bases were determined by leaching the soil with ammonium chloride after the easily soluble salts were removed with water. The pH value was obtained from 1:2 soil:water ratio with the quinhydrone electrode.

SUMMARY

During a rather severe storm in the spring of 1932 a portion of the bayside area of Accomac County, Virginia, including several thousand acres of farm land was flooded with salt water.

The soils that were freshly cultivated before the flooding took up much more sodium chloride than did the soils that had not been cultivated.

Considerable replaceable calcium was removed from the soil, whereas magnesium was increased somewhat by the flooding of the soil.

The sodium chloride content of the surface 0–3⅓-inch soil horizon increased during dry periods and decreased during rainy periods throughout the summer.

About 0.2 per cent sodium chloride was found to be serious on the light sandy soils, whereas greater concentrations did not kill strawberry plants outright on the soils high in organic matter.

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THE EFFECTS OF ADDITIONS OF CERTAIN COLORADO SOILS UPON THE OUTGO OF BASES, CHLORIDES, AND SULFATES FROM A TENNESSEE SOIL

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In a previously cited series of publications (4) by Headden and by Sackett, it was shown that the localized infertile areas, or "brown spots," that occur in certain Colorado soils are characterized by abnormal occurrences of nitrates, chlorides, and sulfates of calcium, magnesium, sodium, and potassium. The accumulation of nitrates was attributed to the activities of nitrogen-fixing, ammonifying, and nitrifying organisms. This explanation was not accepted universally, since it failed to account for the concomitant accumulation of chlorides and sulfates.

During a visit to the Colorado station it occurred to us that it would be of value to determine how these particular Colorado soils would behave under Tennessee conditions and whether substantial additions of the Colorado soils would impart their characteristic properties to Tennessee soils. Accordingly, a 4-year lysimeter study of two of the Colorado soils and their 1 per cent admixtures with a Cumberland clay loam was carried out. The primary objective was to determine whether the postulated bacterial effects would continue under Tennessee conditions and whether the peculiar bacterial activities of the Colorado soils would be imparted to a representative Tennessee soil. It was believed essential, however, to have a complete record of the behavior of the other ions that had accumulated along with the nitrate radical. The outgo of ammonia, nitrites, and nitrates, supplemented by Kjeldahl determinations on the original and the residual soils, has been reported (4). The rapidity and extent of outgo of the components, other than nitrates, from the Colorado soils and the accelerative and the repressive effects exerted by their admixtures with the Tennessee soil, with and without certain supplemental treatments, are given in the present contribution.

EXPERIMENTAL

Procedure and treatment

One hundred pounds of soil, moisture-free basis, was used for the controls of the Grand Junction, Rocky Ford, and Cumberland clay loam soils, whereas each soil combination consisted of 100 pounds of the Cumberland clay loam and 1 pound of a Colorado soil. No stratum of subsoil was used in the tanks, which were $\frac{1}{20,000}$ acre in area. The mixtures of untreated soils were run in parallel with those that received supplemental treatments; (*a*) 100-mesh, high-calcic limestone, (*b*) red clover hay, (*c*) limestone and red clover hay, and (*d*) limestone, red clover hay, and sodium chloride. The heavy limestone treatment, equivalent to a rate of 3 tons of CaO, was made to insure that the organisms carried by the Colorado soils would be given a medium similar to their native habitat. Since a fixation of nitrogen would involve available energy, the large additions of organic matter were made to care for this factor and to furnish oxidizable forms of nitrogen and sulfur. Since one of the Colorado soils contained large quantities of chlorides, a definite supplemental supply of sodium chloride was added to one group of the mixtures.

The soils were maintained fallow, without stirring, during the full period of the experiment. To insure opportunity for the added limestone to react with the Tennessee soil and for the bacterial flora of the added soils to become "acclimatized" in the new medium, a preliminary period of 70 days was permitted between the date when soil additions and treatments were mixed and the beginning of the 4-year period of leaching.

Analysis of leachings

The rainfall for the four years, in order, was 58.97, 51.06, 41.92, and 44.50 inches. During that period 31 collections of rainwater leachings were obtained. These separate collections were analyzed for ammonia, nitrites, nitrates, chlorides, and bicarbonates, and for pH values. The remaining components were determined upon the annual composites. The pH values will not be given in detail. In general, however, they were in excess of 7.5 for all combinations, the original pH values of the Tennessee, Grand Junction, and Rocky Ford soils being, 6.7, 7.3, and 7.5, respectively.

Chemical and mechanical analyses of soils

The chemical analyses of the three soils are given in table 1. The amounts of the several components were determined by extractions with water and with 0.02 N HCl and by HCl, 1.115 s.g., digestions. The aqueous extractions were made by agitating 50 gm. of soil with 200 cc. of water for 1 hour, filtering on a 9-cm. Büchner, and washing to a volume of 1 liter. The chlorine determinations, reported in two instances as "traces" in the per cent columns, were 16 p.p.m., 21 p.p.m., and 12,414 p.p.m. for the Tennessee, Rocky Ford, and Grand Junction soils, respectively. The 0.02 N acid extraction was obtained by first adding to a 50-gm. charge of soil the computed amount of HCl required to neutralize the carbonates and agitating overnight, followed by filtration on a 9-cm. Büchner and a supplemental leaching with 0.02 N HCl to a volume of 1 liter. The computation for "exchangeable" Ca + Mg was obtained by deducting from the total Ca + Mg extracted by the preliminary digestion and the 0.02 *N* HCl extraction, the sums of the corresponding water-soluble and carbonate values.

The Tennessee soil was a carbonate-free, slightly acid, residual soil from a dolomitic limestone formation and had a low content of bases. Both Colorado soils were strongly calcareous; comparable in their total contents of calcium and magnesium, but not in the amounts soluble in either water or dilute HCl.

TABLE 1

Chemical analyses of Tennessee and Colorado soils

	TENNESSEE	GRAND JUNCTION	ROCKY FORD
	per cent	*per cent*	*per cent*
1.115 HCl digestion:			
Calcium ($CaCO_3 \Bumpeq$)	0.317	6.720	4.410
Magnesium ($CaCO_3 \Bumpeq$)	0.611	4.854	3.080
Potassium (K)	0.258	0.450	0.714
Sodium (Na)	0.034	0.658	0.059
0.02N HCl leaching:			
Calcium ($CaCO_3 \Bumpeq$)	0.208	6.815	4.409
Magnesium ($CaCO_3 \Bumpeq$)	0.116	3.775	0.767
Potassium (K)	0.025	0.082	0.031
Sodium (Na)	0.001	0.464	0.016
CO_2 ($CaCO_3 \Bumpeq$)	0.011	7.730	3.320
Water extractable:			
Calcium ($CaCO_3 \Bumpeq$)	0.027	0.815	0.149
Magnesium ($CaCO_3 \Bumpeq$)	0.038	0.685	0.087
Potassium (K)	0.008	0.047	0.015
Sodium (Na)	0.000	0.445	0.013
Sulfates (SO_3)	0.014	0.377	0.030
Nitrates (N)	0.003	0.054	0.011
Chlorides (Cl)	tr.†	0.012	tr.‡
Exchangeable Ca + Mg ($CaCO_3 \Bumpeq$)*	0.248	1.360	1.620

* Computed by subtracting water-soluble + carbonate values from the Ca + Mg soluble in 0.02*N* HCl.

† 16 p.p.m.

‡ 21 p.p.m.

The Grand Junction soil contained much the larger quantities of salts and considerable quantities of either undisintegrated dolomite or high magnesic limestone, whereas a large fraction of the acid-soluble magnesium of the Rocky Ford soil was in the form of silicates.

On the basis of mechanical analyses by the Bouyoucos method (1), the Tennessee soil was classified as a clay, the Grand Junction soil as a sandy loam, and the Rocky Ford soil as a clay. The respective "colloid" contents by that method were 42.4 per cent, 24.4 per cent, and 38.1 per cent. The corresponding "sand" contents were 34.6 per cent, 52.8 per cent, and 40.4 per cent, giving "silt" occurrences, of 30.5 per cent, 24.5 per cent, and 27.9 per cent by difference. It was necessary first to remove the 2.4 per cent content of water-soluble matter from the Grand Junction soil to effect dispersion, but the water-soluble content of 0.2 per cent was not sufficient to vitiate the results obtained when the unleached Rocky Ford soil was dispersed.

OUTGO OF CALCIUM AND MAGNESIUM

The large content of water-soluble salts of calcium and of magnesium shown for the Grand Junction soil in table 1 is reflected by the data of table 2 which show the heavy outgo of these two elements from the full charge of that soil. About one-eighth of its total calcium content was water-soluble and 83 per cent of its total outgo of calcium was obtained during the first year, as against 94.6 per cent for magnesium. The total loss of calcium from the Rocky Ford soil greatly exceeded the loss of magnesium and was about a third as much as the calcium outgo from the Grand Junction soil. The outgo of magnesium from the Rocky Ford soil was about 15 per cent as much as that from the Grand Junction soil. The total outgo of calcium from the Grand Junction soil was less than the amount found by aqueous extractions, whereas the amount of magnesium leached during the first year was greater than the amount obtained in the laboratory extraction. On the other hand, the amounts of calcium and magnesium leached from the Rocky Ford soil were materially in excess of the respective amounts found by aqueous extraction. The total outgo of magnesium was greater than that of calcium because of the difference found for the first year. Hence, under natural leaching, the mobility of the magnesium salts reverses the relation shown for the Grand Junction soil by the aqueous extraction, which gave more calcium than magnesium.

It will be noted that the ratios for the total calcium and magnesium contents of the two soils are almost identical, but the ratios for the water-soluble fractions differ materially. After deduction of the water-soluble fractions from the amount soluble in dilute acid, the ratio for the Grand Junction soil is 2:1 as against 4.8:1 for the Rocky Ford soil. In the former soil a greater fraction of the magnesium occurs in the dolomitic combination, whereas the Rocky Ford soil contains a larger proportion of silicates.

Increments of 163 pounds and 30 pounds, $CaCO_3 \Leftrightarrow$, of water-soluble calcium were supplied by the respective 1 per cent additions of Grand Junction and Rocky Ford soils. Each soil addition, with or without the supplemental addition of calcium, either as limestone or as the water-soluble forms in the clover hay, caused an increase in the amount of calcium leached. When added alone, limestone caused comparable increases of nearly 3,000 pounds $CaCO_3 \Leftrightarrow$ in the outgo of calcium.

The single additions of clover hay in the third group caused a 495-pound $CaCO_3 \Leftrightarrow$ increase in the outgo of calcium from the Tennessee soil, and 567

TABLE 2

Outgo of calcium and magnesium from a Tennessee Cumberland loam with a constant addition of 1 per cent of Colorado soils, with and without supplements of limestone, red clover hay,† and sodium chloride‡ during a 4-year period*

SOILS AND TREATMENT	$CaCO_3 \Leftrightarrow$ PER 2,000,000 POUNDS OF SOIL, MOISTURE-FREE BASIS									
	Calcium					Magnesium				
	'28–29	'29–30	'30–31	'31–32	'28–32	'28–29	'29–30	'30–31	'31–32	'28–32
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Tennessee alone	539	313	232	230	1,314	214	153	91	80	538
Tennessee + Grand Junction	712	314	208	208	1,442	384	199	126	114	823
Tennessee + Rocky Ford	642	318	244	236	1,440	240	152	74	78	544
Tennessee + limestone	1,947	1,157	598	545	4,247	217	128	47	41	433
Tennessee + limestone + Grand Junction	1,998	1,153	639	606	4,396	268	136	49	46	499
Tennessee + limestone + Rocky Ford	1,915	1,250	657	600	4,422	251	130	42	44	467
Tennessee + clover hay	825	424	299	261	1,809	428	206	118	104	856
Tennessee + clover hay + Grand Junction	1,051	331	324	303	2,009	642	230	165	161	1,198
Tennessee + clover hay + Rocky Ford	923	418	312	291	1,944	469	222	119	113	923
Tennessee + clover hay + limestone	2,369	1,166	630	644	4,809	455	171	56	57	739
Tennessee + clover hay + limestone + Grand Junction	2,524	1,192	730	631	5,077	553	195	71	65	884
Tennessee + clover hay + limestone + Rocky Ford	2,448	1,234	697	683	5,062	497	165	59	56	777
Tennessee + hay + limestone + sodium chloride	2,317	1,188	694	642	4,841	465	190	66	52	773
Tennessee + hay + limestone + NaCl + Grand Junction	2,584	1,194	716	675	5,169	571	189	71	63	894
Tennessee + hay + limestone + NaCl + Rocky Ford	2,306	1,260	673	676	4,915	470	192	60	51	773
Grand Junction soil alone	12,457	1,285	582	601	14,925	15,798	472	213	202	16,685
Rocky Ford soil alone	2,401	1,171	555	588	4,715	1,418	625	252	244	2,539

* 3 tons $CaO \Leftrightarrow$, high-calcium stone.

† 12 tons, moisture-free basis, carrying 435 pounds of K.

‡ 500 pounds NaCl.

pounds and 504 pounds for the respective additions of the Grand Junction and Rocky Ford soils. The larger losses shown for the two added soils are more than accounted for by the amounts of water-soluble calcium introduced by them. Moreover, the maximal increase was only 60 per cent of the 941-pound $CaCO_3 \Bumpeq$ increment furnished by the clover hay, 156 pounds of this 941-pound increment being initially water-soluble. The increases in calcium outgo caused by the clover hay alone were decidedly less than those caused by limestone alone, but in each instance the use of clover hay with limestone resulted in a still further enhancement in the outgo of calcium from the limestone treatments. Again, as in the case of clover hay supplements without limestone, these enhancements are substantially less than the amounts of calcium introduced by the clover. The sodium chloride exerted no apparent effect upon the outgo of calcium.

The addition of the Grand Junction soil, of high water-soluble magnesium content, materially increased the amount of magnesium leached from the Tennessee soil, otherwise untreated. But this enhancement of magnesium outgo from the increments was changed to a decided diminution by the limestone supplement, the outgo of magnesium from each unit of the second, or limestone, group being very much less than from each corresponding unlimed unit of the first group.

A decided increase in magnesium outgo was caused by the single clover hay addition that carried a $CaCO_3 \Bumpeq$ of 552 pounds of magnesium, 235 pounds of which was initially water-soluble. But when the third group, which received clover hay without limestone, is compared with the fourth and fifth groups, which received both clover hay and limestone, it is evident that the outgo of magnesium was again definitely depressed by the limestone, in spite of increments supplied by the clover hay. This demonstrates that the repressive, or protective, effect exerted by high-calcic limestone upon magnesium solubility, which has been reported for other Tennessee soils (2), is registered not only by the natural magnesium content of the Tennessee soil, but likewise by the diminished solubility of the substantial quantities of soluble magnesium that were supplied by the additions of both of the Colorado soils and also by the amounts introduced through the clover hay supplements. The sodium chloride addition exerted no effect upon the outgo of magnesium.

In each of the five groups, the outgo of both calcium and magnesium showed a decided decrease after the maximal losses of the first year. Further decreases were registered in the third year, but in general the several losses for the third and fourth years were about equal. The rainfall was greatest during the first year, next highest for the second year, and about equal for the third and fourth years.

OUTGO OF SODIUM AND POTASSIUM

The data of table 3 show that the excessive content of sodium salts in the Grand Junction soil was quickly removed. Of the 10,024-pound outgo of

TABLE 3

Outgo of sodium and potassium from a Tennessee Cumberland loam with a constant addition of 1 per cent of Colorado soils, with and without supplements of limestone, red clover hay,† and sodium chloride‡ during a 4-year period*

SOILS AND TREATMENT	SODIUM AND POTASSIUM PER 2,000,000 POUNDS OF SOIL, MOISTURE-FREE BASIS									
	Sodium as Na					Potassium as K				
	'28–29	'29–30	'30–31	'31–32	'28–32	'28–29	'29–30	'30–31	'31–32	'28–32
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Tennessee alone	40	11	9	5	65	79	65	25	26	195
Tennessee + Grand Junction	127	19	11	9	166	72	50	20	23	165
Tennessee + Rocky Ford	48	11	8	5	72	85	58	22	24	189
Tennessee + limestone	38	13	8	5	64	46	36	19	17	118
Tennessee + limestone + Grand Junction	120	17	9	8	154	37	35	18	18	108
Tennessee + limestone + Rocky Ford	36	13	6	4	59	52	35	19	19	125
Tennessee + clover hay	45	14	8	5	72	208	114	52	46	420
Tennessee + clover hay + Grand Junction	132	15	9	8	164	185	88	50	52	375
Tennessee + clover hay + Rocky Ford	49	12	8	5	74	215	114	52	47	428
Tennessee + clover hay + limestone	44	12	7	5	68	158	98	39	38	333
Tennessee + clover hay + limestone + Grand Junction	128	16	7	6	157	182	102	45	40	369
Tennessee + clover hay + limestone + Rocky Ford	44	10	6	5	65	199	95	40	42	376
Tennessee + hay + limestone + sodium chloride	228	15	7	5	255	188	94	43	40	365
Tennessee + hay + limestone + NaCl + Grand Junction	327	15	8	7	357	224	91	43	47	405
Tennessee + hay + limestone + NaCl + Rocky Ford	219	14	8	5	246	177	96	41	39	353
Grand Junction soil alone	9,846	85	45	48	10,024	672	88	46	50	856
Rocky Ford soil alone	336	28	11	9	384	133	74	36	35	278

3-ton CaO≎.
12 tons, moistu
500 pounds.

sodium from the full charge of the Grand Junction soil, 98.2 per cent was obtained during the first year. Neither the clover hay nor the limestone seemed to affect the consistent increase in sodium outgo induced by the additions of the Grand Junction soil in the several combinations, whereas the 3-pound increment of sodium from the Rocky Ford soil was not sufficient to be registered by the leachings. It will be noted that in each instance, the largest quantity of sodium leached was obtained during the first year, and that all of the several combinations were at equilibria during the third and fourth years. The average sodium outgo from the units of the fifth, or sodium chloride group, gives a 189-pound sodium recovery from the 197-pound addition. Under these experimental conditions, the limestone did not exert for soluble sodium salts the repressive effect upon solubility that will be shown for both the native supplies of potash and those carried by the additions.

The Grand Junction soil was rich in available potassium, 940 pounds water-soluble and 1,640 pounds soluble in dilute HCl. Hence, there was obtained a total outgo of 856 pounds, 672 pounds of which were obtained during the first year. This soil was also strongly alkaline, and, in spite of the amount of readily available potassium supplied by the 1 per cent additions, these additions materially decreased the outgo of potassium from the otherwise untreated Tennessee soil. This repressive effect upon potassium outgo is also shown to a still greater extent in the Grand Junction-Cumberland combination that was treated with limestone alone. The solubility of potassium in the Cumberland loam and also that in its admixtures with the Rocky Ford addition were likewise depressed by the limestone treatments. This is in harmony with previous lysimeter results (3). The Rocky Ford soil was not sufficiently high in available potassium, nor sufficiently alkaline, to register *per se* the effect shown by the more potent Grand Junction soil.

The potassium increment from the clover hay additions was 435 pounds in all units of the third, fourth, and fifth groups. The recovery from the amount supplied to the controls of the Tennessee soil by the clover hay was 51.7 per cent of the addition. This recovery was reduced 45 pounds by the addition of the Grand Junction soil, in spite of its high content of available potassium, and unaffected by the Rocky Ford soil. When added to the Tennessee soil with clover hay, limestone decreased the potassium recovery 87 pounds below the amount obtained from the clover hay alone. The alkalinity of the units of the fourth group that received both the heavy limestone treatment and clover hay was not materially increased by the additions of the Grand Junction soil, but the increments of soluble and exchangeable potassium were increased; hence, the potassium outgo from the unit that received the Colorado soil, together with limestone and clover was in excess of that from the controls, in both the fourth and fifth groups.

When added along with limestone and clover hay, the sodium chloride caused a somewhat greater potassium outgo from the Tennessee soil that received only limestone and clover hay. This was true also of the units that received the additions of the Grand Junction soil. In both cases the increases were obtained during the first year. This effect was not shown, however, for the Rocky Ford soil. The greater outgo of potassium induced by the sodium chloride cannot be attributed to a simple and lasting interchange of sodium for potassium, because an almost complete recovery of the added sodium was obtained during the first year.

OUTGO OF SULFATES AND CHLORIDES

The annual and total losses of sulfates and chlorides are given in table 4. The full charge of the Grand Junction soil gave an outgo of sulfates almost seven times as great as the 1,079-pound outgo obtained from the Rocky Ford soil and over 16 times as great as that from the untreated Tennessee soil. This excessive sulfate increment was readily soluble, 92.5 per cent of the total outgo having been obtained during the first year. An increased sulfate outgo was induced by each Grand Junction addition in all five series. The increases were accounted for almost entirely by the results found for the year. The additions of the Rocky Ford soil also increased the sulfate outgo in each group. The increases were small, however, in the second and fourth groups and not so extensive as those found for the Grand Junction soil in the other comparisons.

When used alone, limestone caused substantial increases in sulfate leachings. The effect of the limestone treatment was much greater than that found for either of the two sulfur-carrying soil additions. In a comparison of the three units of the second group with the corresponding units of the third group, it is evident that the sulfofication effect of the limestone was greater than the sum of any bacterial effect and additive factor attributable to the unsupplemented cover hay, although the hay supplied 138 pounds of SO_3, of which 78 pounds was water-soluble. The combination treatment of clover hay and limestone, with and without the sodium chloride supplements, gave a still greater sulfate outgo than that found for the first three series—no supplements, limestone, and limestone plus clover hay. Since the limestone treatments uniformly produced a marked increase in sulfate outgo, regardless of either soil additions or sulfur increments, the limestone treatments definitely influenced one or both of the factors, leachability and sulfofication.

The results in table 4 show that the total outgo of chlorine from the full charge of Grand Junction soil was 26,647 pounds. Of this, all except 30 pounds was obtained during the first year, 24,759 pounds going out in the leachings of the first two months. During the third year, the losses from the full charges of both Colorado soils were at the level found for the several admixtures of the Tennessee soil. The chlorine results were obtained for only three annual periods, since, after the second year, the losses were uniform for all conditions. It is evident that, with the 50-inch rainfall that occurs at the Tennessee station, and with adequate drainage, the Grand Junction soil would not have accumulated a high chlorine content.

In each intra-comparison for all five groups, the Grand Junction soil pro-

TABLE

Outgo of sulfates and chlorides from a Tennessee Cumberland loam with a constant addition of 1 per cent of Colorado soils, with and without supplements of limestone, red clover hay,† and sodium chloride‡ during a 4-year period*

SOILS AND TREATMENT	SULFATES AND CHLORIDES PER 2,000,000 POUNDS OF SOIL, MOISTURE-FREE BASIS								
	SO₃					Chlorine			
	'28 29	'29–30	'30–31	'31–32	'28–32	'28–29	'29–30	'30–31	'28–31§
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Tennessee soil alone	173	141	52	87	453	60	4	5	69
Tennessee + Grand Junction	272	131	57	94	554	304	5	4	313
Tennessee + Rocky Ford soil	237	135	56	98	526	55	5	4	64
Tennessee + limestone	354	139	97	100	690	59	10	5	74
Tennessee + limestone + Grand Junction	400	135	97	100	732	289	15	4	308
Tennessee + limestone + Rocky Ford	356	139	103	101	699	58	10	4	72
Tennessee + clover hay	194	167	69	89	519	99	5	6	110
Tennessee + clover hay + Grand Junction	283	181	83	108	655	329	7	3	339
Tennessee + clover hay + Rocky Ford	242	173	72	107	594	104	5	6	115
Tennessee + clover hay + limestone	357	157	109	109	732	103	10	5	118
Tennessee + clover hay + limestone + Grand Junction	407	157	111	97	772	354	13	7	374
Tennessee + clover hay + limestone + Rocky Ford	358	160	111	109	738	108	9	5	122
Tennessee + hay + limestone + sodium chloride	340	154	109	105	708	384	12	5	401
Tennessee + hay + limestone + NaCl + Grand Junction	420	161	113	109	803	643	11	4	658
Tennessee + hay + limestone + NaCl + Rocky Ford	341	165	117	108	731	383	12	7	402
Grand Junction soil alone	6,960	339	117	110	7,526	26,617	25	5	26,647
Rocky Ford soil alone	712	156	106	103	1,079	123	7	3	133

3 tons CaO≈.

12 tons, moisture-f basis.

500 pounds NaCl.

Chloride additions npletely removed.

duced a decided increase an average of 244 pounds, in chlorine outgo. The Rocky Ford soil did not augment the chlorine outgo, as would be expected because of the meager increment furnished by the 1 per cent addition. In each instance, practically the full enhancement in chlorine outgo from the combination units is accounted for by the increase found during the first year. The chlorine recoveries from the 500-pound sodium chloride addition to the fifth group were 283, 284, and 280 pounds, for the Tennessee soil, and the Grand Junction and the Rocky Ford combinations, respectively. The average of these three closely agreeing results represents a 93 per cent recovery of the added chlorine, as against a 96 per cent recovery of the sodium content of the added sodium chloride.

SUMMARY AND CONCLUSION

Two "brown-spot" calcareous Colorado soils, rich in accumulated salts and exchangeable bases, were used in a 4-year lysimeter experiment to determine their behavior under Tennessee conditions, and to determine capacities of 1 per cent additions to impart their bacterial and chemical characteristics to a Cumberland clay loam in its natural state and also when modified by supplemental treatments of limestone, red clover hay, and sodium chloride.

As had been found true for nitrates (4), the outgo of sodium and chlorine from the Colorado soils was reduced almost to the level found for the Tennessee soil, mainly during the first year and even in the first collection of leachings. Although the accumulations of calcium and magnesium were speedily and materially reduced, the outgo from both soils continued substantially in excess of that found for the Tennessee soil. The total quantities of magnesium leached from all three soils were considerably in excess of the respective quantities obtained by aqueous extractions.

The additions of both of the Colorado soils caused a 9.6 per cent increase in the outgo of calcium, which was much less than the effect produced by red clover hay, and only a fraction of the three-fold increase found for the limestone additions.

Outgo of magnesium was materially increased by the addition of the Grand Junction soil, in all of the groups. For the Rocky Ford soil, however, this was not true after the first year. Magnesium outgo was uniformally and materially increased by the clover hay, uniformally and materially depressed by limestone, and unaffected by the sodium chloride additions.

The increases in outgo of both calcium and magnesium from the clover hay additions were less than the increments carried by the additions. The additions of the Grand Junction soil caused a uniform increase in total outgo of sodium, and the several combinations reached a common level after the second year. Approximately 95 per cent of the added sodium chloride was recovered.

In spite of its high content of soluble potassium, the Grand Junction soil additions materially depressed the outgo of potassium, whereas the Rocky

Ford addition did not. This repressive effect upon potassium outgo was further increased by the limestone treatments. No replacement of potassium was induced by the sodium chloride in any of the three alkaline combinations, although outgo was accelerated in two instances.

The heavy accumulations of sodium were almost completely removed from the Grand Junction soil during the first year. The sodium carried by the sodium chloride additions was recovered almost completely and uniformly from all combinations, without any apparent effect upon the outgo of either calcium or magnesium.

Sulphate outgo was increased by the additions of each of the Colorado soils and by clover hay, but in neither case was the effect so great as that induced by limestone; hence, the combined effects of addition, inoculation, and alkalinity of the added soils were less than the effect of limestone upon the factors of sulfofication and mobility of sulfates.

Chlorine outgo was uniformally increased by the Grand Junction additions and unaffected by those of the Rocky Ford soils.

The abnormal bacterial characteristics of the Colorado soils were not maintained under the imposed conditions, nor were they imparted to the Tennessee soil in either its natural or its modified state. In general, the limestone and clover hay supplements exerted more effect than did the soil additions. The high salt content and the alkaline nature of the additions did register in the solute contents of the leachings from the several combinations. No base interchange was in evidence, whereas "reciprocal repression" was definitely registered for the 1 per cent addition of the Colorado soils.

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A METHOD FOR THE CONTINUOUS AUTOMATIC EXTRACTION OF SOILS[1]

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The purpose of this paper is to describe a method used at the Nebraska Station for the extraction of soils in base exchange and soluble constituent studies. It will be obvious that several of its features are borrowed, but, on the whole, it is original so far as the writer is aware.

A single extraction unit is shown in figure 1. *A* is a percolator of some sort, for convenience a 35-mm. filter tube which takes a No. 7 rubber stopper. One hole of the stopper bears a short siphon delivery tube and the other an adjustable solid glass rod. *B* is a levelling tube consisting of a length of 7-mm. glass tubing connected to *A* by a length of rubber tubing bearing a pinch clamp. *C* is a constant level reservoir consisting of a flask inverted in a suitable beaker. *D* is a calibrated siphon of two glass pieces connected with rubber. In our laboratory 12 extraction units are assembled in a rack that has one shelf 40 inches above the table and another 30 inches higher. The units are attached with burette clamps to rods spaced 6 inches apart.

A single percolator is filled as follows. In the bottom is a small circle of filter paper wet with a drop of water, and on it a layer of quartz sand of depth sufficient to fill the rounded portion of the tube. Above the quartz sand is the soil sample, compacted, if moist, with the blade of a spatula and then levelled off with a flat-end pestle. Above the soil is 3 to 10 mm. more of quartz sand, and then a perforated porcelain plate held tightly in place by adjusting the siphon and rod after insertion of the stopper.

The reservoir flask having been filled with extractive, the siphon is started and after entrapped air bubbles are all expelled from the levelling tube the pinch clamp is opened. The liquid ascending evenly through the soil finally comes to drip from the delivery tube at the same rate that it siphons from the reservoir, the height of the liquid in the levelling tube automatically adjusting itself to the permeability of the sample. The top of the levelling tube should stand above the level of the liquid in the beaker to prevent overflow in case the sample becomes impervious. In our laboratory siphons are ordinarily calibrated to deliver about 1 cc. per minute. For this rate a 30-inch level tube generally suffices, though a 60-inch tube occasionally is necessary.

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[1] Contribution from the department of agronomy, published with the approval of the director as Paper No. 134 Journal Series.

[2] Professor of agronomy.

For percolators, the ordinary filter tube is very satisfactory. The 28-mm. tube takes a 20-gm. sample, and the 35-mm. tube a 40-gm. sample. Where larger quantities of soil are to be percolated, longer rather than wider tubes should be employed.

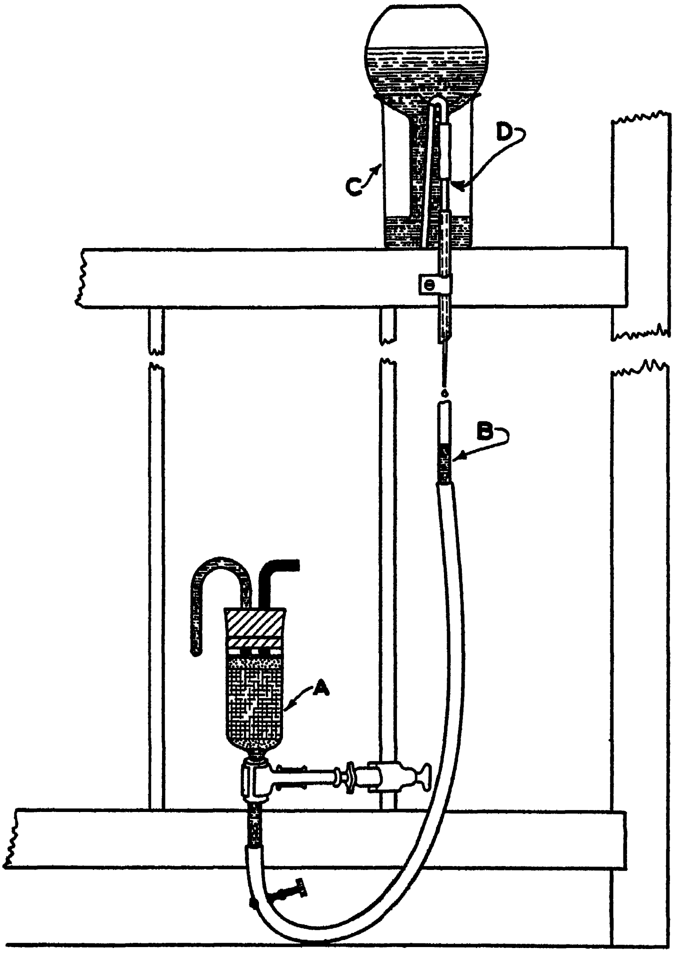

FIG. 1. SECTIONAL VIEW OF A SINGLE EXTRACTION UNIT

The method has been used for base exchange extraction, soil solution for colorimetric determination of pH, nitrates, chlorides, water-soluble phosphorus, phosphorus by extraction with 0.001 *N* sulfuric acid, and water-soluble constituents generally.

With CO_2-free water, clear leachates are not always obtainable. Dried samples give much clearer leachates than the same soils in moist condition. Saline soils are particularly troublesome and not only give muddy leachates but

TABLE 1

Quantity of leachate required for complete extraction of chlorides from two silt loam soils

SOIL 1			SOIL 2		
Volume of leachate	Chlorine in leachate		Volume of leachate	Chlorine in leachate	
	Milli-grams	Per cent of total		Milli-grams	Per cent of total
40-gm. field-moist sample					
First 8.5-cc. portion*	6.95	79.9	First 10.2-cc. portion*	16.20	83.7
Second 8.5-cc. portion	1.50	17.2	Second 10.2-cc. portion	3.10	16.1
17–50 cc	0.25	2.9	20.4–50 cc	0.05	0.2
50–100 cc	0.00	0.0	50–100 cc	0.00	0.0
Total	8.70	100.0	Total	19.30	100.0
0–25 cc	8.95	100.0	0–25 cc	19.65	100.0
25–50 cc	0.00	0 0	25–50 cc	0.00	0.0
Total	8.95	100.0	Total	19.65	100.0
40-gm. air-dry sample					
0–25 cc	10.1	74.9	0–25 cc	26.0	96.5
25–50 cc	1.8	15.1	25–50 cc	0.95	3.5
50–100 cc	0.0	0.0	50–100 cc	0.0	0.0
Total	11.9	100.0	Total	26.95	100.0

* The equivalent of the quantity of water in the sample.

TABLE 2

Quantity of leachate required for complete removal of exchangeable calcium and for complete saturation with ammonium

The extractive use was N ammonium acetate, and the soil sample 20-gm. air-dry.*

VOLUME OF LEACHATE COLLECTED	SUSQUEHANNA CLAY	CRETE SILT LOAM	TRIPP SILT LOAM
Exchangeable calcium in m.e. per 100 gm.			
First 125-cc. portion	3.12	12.60	13.10
Second 125-cc. portion	0.38	0.50	0.50
Third 125-cc. portion	0.25	0.25	0.17
Total	3.75	13.40	13.77
Exchange capacity (replaced ammonium) in m.e. per 100 gm.			
250 cc	24.0	18.3	14.6
375 cc	23.8	18.5	14.6

* The data for this table were obtained by Mr. A. R. Marquardt.

become impervious as well, as soon as the bulk of the salts is removed. Anions like Cl^- and NO_3^- can be leached with complete satisfaction from all soils with 0.01 *N* H_2SO_4 or $MgSO_4$. Perfectly clear leachates are obtainable with extractives used in base exchange.

The rapidity with which the non-adsorbed water-soluble constituents are removed is remarkable. Table 1 shows the extraction of chlorides from two silt loam soils. Numerous routine experiences amply corroborate the data of the table not only for chlorides but for nitrates also.

Table 2 shows the efficiency of the method in base exchange procedure. In base exchange routine, a 20-gm. air-dry sample is used. After preliminary leaching, if necessary,[3] with 100 cc. of water (initially CO_2-free), the reservoir, a 250-cc. Pyrex flask in a 200-cc. tall form beaker, is charged with *N* ammonium acetate (pH 7.0), and 250 cc. of leachate is collected for the exchangeable base determinations. As table 2 shows, this quantity fully suffices to remove all replaceable calcium in high calcium soils like Tripp silt loam and also to replace all cations with ammonium in highly unsaturated soils like Susquehanna clay. After the soil has been leached with ammonium acetate, the reservoir and levelling tube are emptied and rinsed, and the reservoir is charged with 250 cc. of 85–95 per cent methyl alcohol for the removal of occluded ammonium. Occasionally 50–100 cc. additional alcohol is required. Finally the reservoir is charged with 250 cc. of *N* calcium chloride and the leachate collected in a Kjeldahl for determination of ammonia by distillation.

The method has been in use for 12 months and has had a thorough trial. It is recommended for its simplicity of equipment and technique, its neatness, its freedom from errors of contamination, and its adaptability to numerous lines of work. Above all, it is recommended for its thoroughness of extraction and its economy of time and chemicals.

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[3] For alkali soils this is a serious problem that has not yet been satisfactorily solved.

THE USE OF INDICATORS FOR THE QUALITATIVE DETERMINATION OF SOIL REACTION

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Information concerning the reaction of soils is important because of the correlation which exists between the "lime need" of certain crops and the intensity of soil acidity. The use of indicators for the determination of soil reaction under field conditions has been proposed by different investigators. Wherry (12) determined the soil reaction by preparing a suspension of soil with distilled water to which indicators were added after the coarser particles had settled. Spurway (11) suggested a method in which an indicator was allowed to percolate through the soil mass and the reaction of the soil was determined by comparing the color of the filtrate with a color chart. Morgan (7) has proposed a similar method in which a porcelain block is used to hold the soil and four different indicators are employed in order to determine the reaction of soils varying from strongly acid to basic in nature. Universal indicators have been prepared by Niklas and Hock (8) and by Kolthoff (5), and a similar indicator has been manufactured for the determination of soil reaction by the LaMotte Chemical Products Company, of Baltimore, Maryland. Universal indicators may be satisfactory for estimating wide differences in degree of acidity or alkalinity, but they are not suitable for the accurate determination of hydrogen-ion concentration.

In comparing the various indicators which have been proposed for the determination of soil reaction, it was found that in many instances good agreement was not obtained between the pH value of the soil as determined electrometrically and by indicator methods. Brom thymol blue, as recommended by Spurway (11), and called "Soiltex," could not be used to differentiate between certain soils which were strong, medium, and slightly acid. Also the color change in the alkaline range was frequently different from the color change of the indicator which occurs in standard buffer solutions. This is one factor which apparently has not been considered by previous investigators in preparing their color charts for the determination of the acidity or alkalinity in soils.

Beaumont and Thayer (1) have made a study of field methods for determining soil reaction and have found that dependable results were obtained when indicator methods recommended by Morgan (7) and Spurway (11) were used.

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[1] Credit is due Harley A. Daniel for assistance in obtaining the data presented in tables 6 and 7 of this paper.

Schollenberger (10) has proposed a method in which the soil is placed in special tubes and is leached with distilled water and the acidity or alkalinity of the filtrate is determined by adding an indicator solution and comparing with a series of standards the color produced. When this procedure is followed, the indicator color change which occurs in standard buffer solution can be used to determine the pH of the soil, provided the reaction of the indicator solution is not appreciably different from that of the soil filtrate. However this method and other similar methods are not applicable for use under field conditions; consequently they have not been studied in connection with this investigation.

EXPERIMENTAL

Since many indicators are acidic in nature and form salts when neutralized with a soluble hydroxide, it is logical to expect that the addition of a neutral indicator solution to a soil would tend to increase the acidity of the soil to an extent similar to that produced when inorganic salts are added to a soil suspension. In the first experiment four different soils were treated with distilled water, with a solution of 0.04 per cent brom cresol purple, and with solutions of 0.01*N* barium chloride, calcium acetate, potassium chloride, and calcium nitrate. In all cases one part of soil was mixed with two parts of liquid. The soil suspensions were shaken intermittently for 20 minutes, and the pH values were determined by means of the quinhydrone electrode. Since this method (quinhydrone) has been subject to criticism under certain conditions (4, 6), the data were omitted where a drift in potential occurred in connection with this study. The results of this experiment are given in table 1.

TABLE 1

A comparison of the effect of 0.01 N *salt solutions and a 0.4 per cent indicator solution of brom cresol purple on the pH value of soils*

SOIL NUMBER	DISTILLED WATER	BROM CRESOL* PURPLE	BARIUM† CHLORIDE	CALCIUM ACETATE	POTASSIUM CHLORIDE	CALCIUM NITRATE
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
4	6.1	5.8	5.0	5.5	5.3	5.1
7	4.8	4.7	4.6	5.0	4.5	4.5
12	6.9	6.6	6.2	6.3	6.4	6.3
19	8.2	7.4	7.6	7.6	7.6	7.6

* pH of indicator 6.3.

† pH of salt solutions 6.1.

Table 1 indicates that decrease in the pH values of the soil suspensions caused by the indicator solution was similar to the effect produced by the addition of inorganic salts. It is interesting to note that the effect of the brom cresol purple solution on the pH of soil 19 was even greater than that produced by the other salts studied.

It was also found that changes in concentration of an indicator solution affect the reaction of soil suspensions. This information was obtained by treating a medium acid soil with brom cresol purple solutions having concentrations of 0.2, 0.04, and 0.004 per cent, and with distilled water as in the previous test. The soil was leached to remove all soluble salts, since Crowther (2) and Pierre (9) have shown that the presence of soluble salts in the soil affect the pH value of the soil suspension. The results of this experiment are given in table 2.

Leaching this particular soil did not appreciably affect the results; although no data were obtained on the salt content of the sample used in this experiment. The indicator solutions were neutralized to pH 6.1 before they were added to the soil. It is quite evident from the data obtained that soils treated with an indicator solution should not agree with the results of distilled water suspensions because of the "salt effect" which the indicator solution has on the soil; although the use of an indicator to which an excess of alkali has been added would tend to offset the salt effect in certain soils.

In order to obtain further information on the pH value of soils as affected by different indicator solutions, 21 soils of varying reaction were selected and comparisons were made using soil suspensions in distilled water and suspensions prepared with .04 per cent solutions of brom cresol purple, brom thymol blue, cresol red, and chlor phenol red. Ten grams of soil and 20 cc. of solution were shaken for 20 minutes, and the pH was determined by means of the quinhydrone electrode. The results of this experiment are given in table 3. Each indicator solution was neutralized with calcium hydroxide to the following pH values before it was added to the soil: chlor phenol red, 5.9; brom cresol purple, 6.1; brom thymol blue, 6.8; cresol red, 8.0. All of these indicators had a pH of approximately 2.4 to 2.5 at a concentration of 0.2 per cent in distilled water.

TABLE 2

Effect of changes in the concentration of brom cresol purple on the reaction of a leached soil

TREATMENT	pH OF SOIL SUSPENSION
Distilled water	5.86
0.2 per cent brom cresol purple	5.54
0.04 per cent brom cresol purple	5.61
0.004 per cent brom cresol purple	5.68
0.04 per cent brom cresol purple (unleached soil)	5.66

The reactions of the soil suspensions in distilled water were usually less acid than the reactions of the soil suspensions with the different indicator solutions. Also the color which is obtained after an indicator solution percolates through a soil may be different from the actual color of the indicator which corresponds to the pH of that soil suspended in distilled water.

Another factor which may affect the use of an indicator in the determination of soil acidity is the amount of absorption which may occur when the indicator passes through the soil. Crowther (2) has pointed out that methyl red is strongly absorbed by soil and cannot be used successfully where the indicator percolates through the soil mass. In order to obtain further information on the absorption of indicators by soils, a comparison of brom cresol purple, brom thymol blue, chlor phenol red, cresol red, and brom cresol green was made (table 4).

It is quite evident that brom thymol blue and brom cresol green are more strongly absorbed than the other three indicators studied. In each case the soil was treated with two parts of 0.04 per cent indicator solution, and the amount of adsorption was determined by diluting 5 cc. of the clear filtrate with 50 cc. of 0.1 per cent hydrochloric acid, which was compared in a Dubosc colorimeter with 5 cc. of the original indicator solution treated in a similar manner. The absorption of the indicator is more closely associated with the clay content of the soil than with any other factor; although certain soils high in organic matter content have been studied which contained very little clay but absorbed large amounts of the indicator from solution.

TABLE 3

A comparison of the effect of indicator solutions on the reaction of soil suspensions

SOIL NUMBER	pH OF SOIL SUSPENSION				
	Distilled water	0.04 per cent brom cresol purple	0.04 per cent brom thymol blue	0.04 per cent cresol red	0.04 per cent chlor phenol red
1	5.23	5.16	5.28	....	5.17
2	7.93	7.74	7.95	7.87	....
3	5.37	5.05	5.26	....	5.21
4	6.23	5.87	6.07	....	....
5	6.34	5.97	6.20	....	....
6	6.23	5.85	5.98	....	....
7	4.87	4.81	4.84	....	....
8	5.84	5.70	5.76	....	....
9	6.89	6.51	6.82	....	....
10	7.17	6.98	6.98	....	....
11	8.28	8.00	7.97	....	....
12	7.15	6.68	7.00	6.96	....
13	6.49	6.36	6.51	....	6.42
14	5.83	5.73	5.84	....	5.73
15	7.17	6.95	7.08	....	7.03
16	8.24	8.00	7.84	8.19	....
17	7.27	6.98	7.15	7.10	....
18	8.71	8.48	8.38	....	....
19	7.98	7.74	7.77	....	....
20	7.86	7.76	7.62	....	7.72
21	7.08	6.66	6.97	6.99	....

Information on the pH values of soil filtrates as compared with the pH values of soil suspensions prepared with indicator solutions was obtained by studying soils adapted to the pH range of the different indicators. Brom cresol green, brom cresol purple, brom thymol blue, and cresol red were used in different concentrations as given in table 5. The pH values of soil suspensions treated with brom cresol green were not appreciably different from those of the soil filtrates; although the more concentrated indicator solution increased the acidity slightly except in case of soil 2638. Clear filtrates could not be obtained from some of the dilute indicator suspensions, and the pH values for these solutions were omitted from the table. Because of differences in concentration the color values of the brom cresol green filtrates were quite different from those which appear when this indicator is added to standard buffer solutions. A better range of color will be obtained with this indicator if a 0.02

TABLE 4

Studies on the absorption of indicators by soils

SOIL NUMBER	pH OF SOIL	PER CENT OF INDICATOR ABSORBED				
		Brom cresol purple	Brom thymol blue	Chlor phenol red	Cresol red	Brom cresol green
1	5.2	6.5	35.0	7.5	....	....
2	7.9	11.5	57.5	....	15.0	....
3	5.4	19.0	49.0	8.0	....	....
4	6.2	12.5	62.5	....	....	....
5	6.3	2.5	30.0	....	....	....
6	6.2	30.0	47.5	....	....	....
7	4.9	27.5	57.5	....	....	....
8	5.8	32.5	77.5	....	....	....
9	6.9	22.5	80.0	....	....	....
10	7.2	36.5	57.5	....	....	....
11	8.3	1.5	49.0	....	....	....
12	7.1	2.5	30.0	....	6.5	....
13	6.5	17.5	57.5	10.0	....	....
14	5.8	18.5	40.0	5.0	....	....
15	7.2	16.0	52.5	10.0	....	....
16	8.2	9.0	30.0	....	6.5	....
17	7.3	7.5	27.5	....	2.5	....
18	8.7	7.5	25.0	....	....	....
19	8.0	5.0	21.0	....	....	....
20	7.9	0.0	27.5	6.0	10.5	....
21	7.1	20.0	42.5	....	12.5	....
699	4.8	....	....	....	....	32.5
947	4.7	....	....	....	....	37.5
1013	4.8	....	....	....	....	20.0
1026	4.6	....	....	....	....	31.5
2632	5.0	....	....	....	....	24.0
2638	4.5	....	....	....	....	50.0
Average per cent absorbed...........		14.5	45.5	7.7	8.9	32.6

per cent solution is used in place of more concentrated solutions even though a considerable amount of this indicator is absorbed when it passes through the soil.

TABLE 5

A comparison of the pH values of soil suspensions and soil filtrates obtained by treating soils with varying concentrations of indicator solutions

SOIL NUMBER	pH OF DISTILLED WATER SUSPENSION	INDICATOR WITH SOIL			FILTRATE FROM SOIL		
		0.4 per cent	0.04 per cent	0.004 per cent	0.4 per cent	0.04 per cent	0.004 per cent
pH values with brom cresol green							
494	4.8	4.6	4.7	4.7	4.5	4.9	..*
947	4.7	4.5	4.8	4.8	4.5	4.8	..*
1013	4.8	4.7	4.8	4.8	4.7	4.8	..*
1026	4.6	4.3	4.4	4.4	4.3	4.5	..*
2632	5.0	4.7	4.9	5.0	4.7	4.8	..*
2638	4.5	4.6	4.5	4.5	4.6	4.7	..*
pH values with brom cresol purple							
1	5.2	5.0	5.2	5.4	4.6	..*	..*
2	7.9	7.4	7.7	7.9	6.4	6.6	..*
5	6.3	5.8	6.0	6.3	5.5	5.8	..*
7	4.9	4.8	4.8	4.9	4.7	5.0	..*
14	5.8	5.5	5.7	5.7	5.4	5.6	..*
21	7.1	6.4	6.7	6.6	6.0	6.3	6.4
pH values with brom thymol blue							
1	5.2	5.2	5.3	5.3	5.4	5.5	5.6
2	7.9	7.6	7.9	7.9	6.8	6.8	6.9
5	6.3	6.2	6.2	6.4	5.7	6.0	6.0
7	4.9	4.8	4.8	5.0	5.0	5.2	5.5
14	5.8	5.8	5.8	6.1	5.7	5.8	6.0
21	7.1	7.0	7.0	7.1	6.4	6.4	6.7
pH values with cresol red							
2	7.9	7.8	7.9	8.1	6.7	7.0	7.1
16	8.2	7.9	8.2	8.4	6.7	6.8	7.2
21	7.1	7.0	7.0	7.2	6.6	6.9	6.9

* Solutions could not be filtered free from soil.

In case of brom cresol purple the soil filtrates were much more acid than the soil suspensions. This explains the wide range of pH which this indicator will cover, especially in case of neutral to slightly basic soils, since the color change of soil filtrates obtained in the acid range of the indicator is not appreciably different from that of the soil suspensions. The deviations between the pH values of soil suspensions and filtrates of sandy soils were less than in case of soils containing considerable amounts of silt and clay.

The effect produced by brom thymol blue is quite similar to that produced by brom cresol purple except that it tends to reduce the acidity of soils which have a pH about 5.0 in distilled water. This is probably due to the buffering effect of the indicator, which is similar to the effect produced when neutral calcium acetate is added to the soil.

Cresol red is not adapted to the determination of soil reaction when it is allowed to percolate through the soil mass. Apparently many soils absorb the basic ions from this indicator more rapidly than the indicator solution can dissolve other bases from the soil particles to replace those which have been absorbed, particularly in the alkaline range of the indicator. Further studies on the reactions which occur between different indicator solutions and basic soils are in progress.

In studying the reaction of indicator solutions which were obtained in a commercial set prepared to determine the pH value of soils, it was found that the indicators were all very alkaline as compared with the 50 per cent dissociation constant of each indicator. Since indicators which are not neutralized carefully may give inaccurate results, a study of the effect of the reaction of indicator solutions on the acidity of the soil was made and the results are given in table 6. In this experiment chlor phenol red adjusted to different pH values was compared with the pH values of soils obtained from suspensions in distilled water. The pH values were determined from a color chart giving the color change of this indicator in standard buffer solutions and are comparative although they may not be accurate.

TABLE 6

Studies on the variation in the reaction of soils when extracted with a 0.04 per cent solution of chlor phenol red adjusted to different pH values

SOIL NUMBER	pH OF SOIL IN DISTILLED WATER	pH AS DETERMINED COLORIMETRICALLY BY ALLOWING AN INDICATOR SOLUTION OF DIFFERENT DEGREES OF ACIDITY OR ALKALINITY TO PASS THROUGH THE SOIL					
		Indicator pH 5.1	Indicator pH 5.7	Indicator pH 6.4	Indicator pH 7.0	Indicator pH 7.6	Indicator pH 8.1
107	5.3	5.0	5.1	5.3	5.5	5.7	6.1
148	7.3	5.6	6.0	6.4	6.6+	6.8	6.8*
220	6.0	5.4	5.3	5.9	6.4	6.5	6.6

* Above the color change of the indicator solution.

Since the pH values of these soils vary considerably, it is very evident that the reaction of the indicator solution may play a very important part in the accurate determination of the acidity in the soil. In case of soil 107 and soil 220, the values were below and above the actual acidity as measured by the hydrogen-ion concentration. In case of soil 148 the values were all lower than that obtained by the electrometric determination of pH values by means of the quinhydrone electrode. At a pH of 6.4 the indicator solution gave results which closely approximated those obtained electrometrically. The buffer capacity of different soils would naturally affect the results, the variation being greater in poorly buffered soils than in well-buffered soils.

In a test of various indicators which might be used for determining the soil reaction under Oklahoma conditions, it was found that brom thymol blue, as recommended by Spurway (11), was inaccurate because a good classification of soils could not be made in the region of slight to medium acidity, which is important in determining the lime requirement of soils, particularly for crops like alfalfa and sweet clover. This indicator was also used by Morgan (7), but in his method measurements of soil acidity are restricted to the color changes of the indicator in standard buffer solutions; consequently important errors would only occur in the alkaline range of the indicator.

In order to obtain more accurate information concerning soils which vary in reaction from pH 5.5 to 6.5, brom cresol purple was tried. The transition interval of brom cresol purple in buffered solutions is pH 5.2 to 6.8. It was found, however, that this did not agree with the results obtained when the indicator was permitted to filter through the soil mass and the filtrate was compared with a standard color chart. It was found that most soils which had a pH of 6.8 to 7.2 were still neutral and in some instances were slightly acid according to the colors obtained from a standard color chart for hydrogen-ion concentration, and the full purple color of the indicator was not usually obtained until the reaction of the soil was about pH 7.4 to 7.6. This same condition occurred with brom thymol blue, which did not give a good blue color on many soils until the reaction of the indicator solution was above 7.8. Similar results were also obtained with chlor phenol red.

When water is added to a soil an equilibrium occurs between the solid and liquid phase which is dynamic in nature. When an indicator solution is added to a soil which has an absorptive capacity for bases, the basic ion is taken up by the soil in exchange for hydrogen and an increase in the number of molecules of the acid form of the indicator occurs. In the acid range or the indicator this does not affect the color so much as it does in the basic range of the indicator where a sufficient amount of basic ions must be present in the solution to change the indicator from the iogenic to the pseudo or normal form. Also the solvent effect of the indicator solutions is reduced as the pH value changes from slightly acid to basic, and this may account for some of the results. In this experiment both sodium and calcium indicator salts have been studied and similar effects were produced when indicator solutions neutralized with either of these elements were added to different soils.

Acid soils which are high in organic matter usually give more acid filtrates as measured by their color when treated with indicator solutions than soils having the same pH value but lower in organic matter content. The use of indicator solutions neutralized with sodium hydroxide might tend to reduce this error, since sodium is not absorbed so much as calcium by the organic base exchange complex.

A study of the total amount of titratable bases and calcium obtained when water is passed through the soil and the total amount of titratable bases and calcium obtained in the filtrate from a soil suspension of an indicator solution indicates that either there is a considerable amount of absorption of calcium from the indicator solution or the presence of calcium in the indicator solution represses the solubility of calcium in the soil since the total amount of bases calculated from the amount added with the indicator and the amount extracted from the soil with distilled water is usually greater than the amount of calcium obtained by extracting the soil with an indicator solution. A few of the results obtained in this study are given in table 7.

TABLE 7

Calcium equilibrium between soil suspensions in distilled water and in indicator solutions

SOIL NUMBER	CALCIUM EXTRACTED FROM 100 GM. OF SOIL BY 200 CC. OF SOLUTION			CALCIUM IN 200 CC. OF INDICATOR		ABSORPTION OF INDICATOR BY SOIL		TOTAL Ca EXTRACTED BY INDICATOR SOLUTION MINUS SOLUBLE Ca IN SOIL AND CALCIUM ADDED IN INDICATOR	
	Distilled water	0 04 per cent brom cresol purple	0.04 per cent brom thymol blue	Brom cresol purple	Brom thymol blue	Brom cresol purple	Brom thymol blue	Brom cresol purple	Brom thymol blue
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	*mgm.*	*mgm.*
1	2.8	4.5	6.9	7.68	8.28	6.5	35.0	−5.3	−1.2
2	6.4	12.2	12.5	7.68	8.28	11.5	57.5	−0.9	+2.4
3	2.9	4.7	5.4	7.68	8.28	19.0	49.0	−4.4	−1.6
4	2.6	2.1	4.0	7.68	8.28	12.5	62.5	−8.1	−1.7
5	1.9	4.9	3.8	7.68	8.28	2.5	30.0	−4.4	−3.7
6	2.4	4.5	6.7	7.68	8.28	30.0	47.5	−2.7	−0.4
7	10.7	17.1	20.0	7.68	8.28	27.5	57.5	+0.8	+5.6
9	6.5	7.9	9.4	7.68	8.28	22.5	80.0	−4.5	+1.3
10	4.3	6.8	6.6	7.68	8.28	36.5	57.5	−2.3	−1.2
11	14.6	22.5	20.3	7.68	8.28	1.5	49.0	+2.3	+3.6
12	1.2	6.8	7.4	7.68	8.28	2.5	30.0	−1.9	+0.3

In this experiment the results are rather difficult to analyze, since all calculations were based on the assumption that the calcium content of the indicator absorbed by the soil was the same as that present in the original indicator solution. Of 57 comparisons made, 45 samples contained less calcium than should have been present if no absorption had occurred. Seven of the twelve samples which yielded more calcium according to calculations than that which should have been present are shown in table 7. Similar studies were also conducted with brom cresol green, chlor phenol red, and cresol red. The greatest average difference between the total amount of calcium removed by distilled water plus that added in the indicator solutions and the amount removed by extraction with these indicator solutions was 7.5 mgm. for 100 gm. of soil. This difference was obtained with cresol red and is the average absorption for six soils which were used in the experiment.

METHOD RECOMMENDED

The method used in this laboratory for 6 years and recommended for the qualitative determination of soil reaction is as follows:

> Fold a sheet of aluminum foil[2] lengthwise to form a trough. Place enough soil in one end of the trough to cover an area about ½ inch long and ⅛ inch deep. Add brom cresol purple solution drop by drop to one end of the soil mass. Add one or two more drops after the soil is saturated. Allow solution to remain in contact with soil one minute. Tip the aluminum foil slightly and with the edge of a clean knife blade draw away one drop of clear filtrate from the opposite end of the soil mass to which the test solution was added. Compare color of filtrate removed from soil mass with color chart.

In case of finely powdered soils or compact clays, a thin film of indicator solution removed from the surface of the soil after the indicator has been in contact with the soil for one minute will give relatively accurate results.

The color of soil filtrates from basic soils are bright purple, becoming more reddish as they approach the neutral point. Neutral soils give wine-colored filtrates. Slightly acid soils produce dull greenish yellow filtrates. Medium acid soils yield olive-yellow filtrates, strongly acid soils give bright yellow solutions. The indicator solution is prepared by dissolving 40 mgm. of indicator in 10 cc. of either C. P. methyl or ethyl alcohol. This solution is diluted with 80 cc. of distilled water and adjusted to a pH of 6.1 with a saturated solution of calcium hydroxide. It is then made up to a final volume of 100 cc. with distilled water. The alcohol is used to prevent bacteria and molds from decomposing the indicator. A slight variation in the pH values for different degrees of acidity has been made from that previously reported (3) in order to comply with the experimental results given in table 8.

The aluminum foil which is used in this method does not influence the color change of the indicator and as soon as the test is made the foil can be discarded, which is an important advantage over the method recommended by Morgan (7), in which distilled water must be carried to clean the porcelain block so that subsequent tests can be made. The surface of paraffined paper, as recommended by Spurway (11), is frequently acid and this may affect the results of the test if the solution remains in contact with the soil and paper too long. The method also has advantages over the procedure as recommended by Wherry (12), since a smaller amount of equipment is needed; however the one indicator does not cover a maximum range although it does cover an important range from the standpoint of determining the lime requirement of lime loving crops such as alfalfa, sweet clover, barley, and certain garden crops. Where the test is used in the laboratory some precaution should be used to obtain accurate results, since the indicator is easily affected by acid or ammonia fumes.

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[2] The aluminum foil was obtained from the Aluminum Company of America, Kansas City, Missouri, and the specifications were as follows: Size 1¼ inches x 2½ inches; 0.00065 inch thick, 2 SO foil, embossed pattern A, interleaved with 10 lb. tissue paper. This material can also be obtained in large sheets.

Because of the dichromatism of brom cresol purple, it is not well adapted for use in artificial light; however this dichromatism occurs in the alkaline range of the indicator and from the standpoint of interpreting the results in terms of the lime requirement of soils it is not important. Since brom cresol purple has a much better color change than chlor phenol red and covers approximately the same pH range, it is better adapted to the colorimetric determination of soil reaction than the latter indicator.

TABLE 8

A comparison between the reaction of 1,402 samples of soil as determined by the quinhydrone electrode and by treatment with brom cresol purple

pH VALUE OF SOIL BY QUINHYDRONE ELECTRODE	SOIL REACTION BY BROM CRESOL PURPLE METHOD				pH VALUE OF SOIL BY QUINHYDRONE ELECTRODE	SOIL REACTION BY BROM CRESOL PURPLE METHOD				
	Strongly acid	Medium acid	Slightly acid	Neutral basic		Strongly acid	Medium acid	Slightly acid	Neutral	Basic
4.8 and less	5	..	..	..	6.8	..	..	10	32	..
4.9	5	..	..	..	6.9	..	..	..	36	2
5.0	5	..	..	..	7.0	..	..	1	40	3
5.1	14	..	..	..	7.1	..	..	1	16	6
5.2	14	..	..	..	7.2	..	..	..	19	15
5.3	12	1	..	..	7.3	..	..	..	19	23
5.4	18	7	..	..	7.4	..	..	..	23	21
5.5	18	19	..	..	7.5	..	..	..	6	44
5.6	8	20	1	..	7.6	..	..	..	4	36
5.7	11	38	2	..	7.7	..	..	..	..	35
5.8	4	52	1	..	7.8	..	..	..	..	52
5.9	3	44	2	..	7.9	..	..	..	1	38
6.0	..	44	13	..	8.0	..	..	..	..	29
6.1	..	24	38	1	8.1	..	..	..	..	19
6.2	..	3	47	..	8.2	..	..	..	..	26
6.3	..	1	55	..	8.3	..	..	..	..	21
6.4	1	..	50	..	8.4	..	..	..	..	36
6.5	..	4	53	4	8.5	..	..	..	..	11
6.6	..	1	41	11	8.6	..	..	..	..	34
6.7	..	..	32	27	...	..	..	..	..	..

The correlation between the pH value of soils and the intensity of acidity is similar to the table recommended by Spurway (11) and used by Beaumont (1). A study of 1,402 samples of soil of varying degrees of acidity was made to compare the results of the brom cresol purple method for soil reaction with the pH values of these soils. The results of this comparison are given in table 8. Soils which are basic have a pH value of 7.5 or higher. A deep purple color indicates a more basic reaction than a reddish purple color. Sandy soils usually have a lower pH value when purple colors appear than do soils which contain considerable amounts of silt and clay. Soils which are neutral have a pH value from 6.8 to 7.4. Soils which are slightly acid have a pH value of 6.1 to 6.7. Soils which are medium acid have a pH from 5.5 to 6.0. Soils which are strongly acid have a pH value of 5.4 or less.. There is a slight overlapping of soils, particularly where the different soil reaction groups meet. This would be expected in a qualitative method such as that which has been recommended. Very strong acidity cannot be differentiated from strongly acid soils by this method; however brom cresol green could be conveniently adapted to soils which are very acid by carefully comparing colors obtained with this indicator with the pH values of several different soils using an indicator concentration of .02 per cent in a solution containing 10 per cent methyl alcohol and carefully neutralized to the 50 per cent dissociation constant of the indicator.

SUMMARY

A study was made of several qualitative indicator methods for the determination of soil reaction. It was found that filtrates of indicators passing through different soils did not give the same results as electrometric measurements of the pH values of the soil suspensions.

Since the indicators commonly used are organic acids neutralized with sodium or calcium hydroxide, they should produce a "salt effect" when added to a soil. Data have been presented which show that a reduction in the pH value of soils occurs when indicators are added to them, the effect being more pronounced in slightly acid, neutral, and basic soils.

An increase or decrease in the concentration of the indicator also affects the results. A decrease in the pH value of the soil suspension occurred when the concentration of the indicator was increased, and vice versa.

When different soils were mixed with indicator solutions and filtered, the pH values of the soil filtrates were more acid than the soil suspensions except in case of very strongly acid soils. A study of the absorption of indicators by soils indicated that brom thymol blue and brom cresol green were absorbed to a greater extent than brom cresol purple, chlor phenol red, or cresol red.

A qualitative indicator method for soil reaction has been proposed using brom cresol purple which will separate soils into five groups based on a study of the pH values of 1,402 samples of soil. The pH range of the different groups is as follows:

SOIL REACTION	pH VALUE	COLOR OF FILTRATE
Basic	7.5 or higher	Reddish purple to deep purple
Neutral	6.8 to 7.4	Wine color
Slightly acid	6.1 to 6.7	Dull greenish yellow
Medium acid	5.5 to 6.0	Olive yellow
Strongly acid	5.4 and less	Bright yellow

The solution of brom cresol purple contains 0.04 per cent of indicator and 10 per cent of methyl or ethyl alcohol. It should be carefully neutralized to pH 6.1 with calcium hydroxide. If equipment is not available to measure the pH value, titrate to a light red color.

Brom cresol purple gives sharper color changes than chlor phenol red and is recommended in preference to the latter indicator.

The use of carefully neutralized indicator solutions is necessary in order to obtain accurate information. Further studies on indicators which will give more accurate results with basic soils are being made.

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THE RELATION OF THE NITRIFYING CAPACITY OF SOILS TO THE AVAILABILITY OF AMMONIA AND NITRATES

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When nitrogenous organic matter or ammonium salts are introduced into soils, the nitrogen in them in the course of time is changed to nitrates. Soils vary to a considerable extent in their capacity to change organic matter or ammonium salts to nitrates (1, 4, 5). Aside from the question of the relative values of ammonia and nitrates to plants, which has been extensively studied (3), it is a matter of interest to know whether there is a decided difference between the amounts of ammonia and of nitrates taken up by plants from soils with low nitrifying capacity. It is also of interest to know whether the relative amounts of nitrates and ammonia taken by plants from soils of high nitrifying capacity differ widely from those taken up from soils of low nitrifying capacity.

THE NITRIFYING TESTS AND POT EXPERIMENTS

A number of soils having very low capacities for nitrifying ammonium sulfate together with a few having a high nitrifying capacity, were selected for the pot experiments. In estimating the nitrifying capacity, 0.1 gm. nitrogen in ammonium sulfate was used for 200 gm. of soil, which is equivalent to 500 p.p.m. of nitrogen Water was added, the mixture placed in beakers and incubated at 35°. The loss of water was replaced twice a week. At the end of 4 weeks, nitric and nitrous nitrogen were estimated. The amount of nitric nitrogen produced in the soil without any addition of ammonium sulfate was subtracted from the amount produced in the soil which received ammonium sulfate. The difference gave the amount of nitric nitrogen from the ammonium sulfate. The result was expressed as the percentage of ammonium sulfate nitrified in a standard soil of high nitrifying capacity incubated at the same time and under the same conditions.

In the pot experiments, the soils (5,000 gm. of soil) were placed in galvanized iron pots and 1.0 gm. dicalcium phosphate and 1.0 gm. potassium sulfate added to each pot. Two pots of each set received no further addition, two received ammonium sulfate, and two received nitrate of soda, both equivalent to 0.1 gm. nitrogen in 1931 and 1932. But in 1930, 0.129 gm. nitrogen in sulfate of ammonia and 0.078 gm. nitrogen in nitrate of soda were added. The crop was planted, the pots watered three times weekly, the crops harvested after about 2 months, dried, weighed, and analyzed for nitrogen. The nitrogen in the crops grown without any addition of nitrogen was deducted from the nitrogen in the crops grown with the nitrate of soda or sulfate of ammonia The difference gave the nitrogen recovered from the additions. The final results were calculated to the percentage recovered by the crops from the amount added.

AVAILABILITY OF THE NITROGEN

The results of the work are summarized in table 1. Details of some of the experiments are given in table 2, but it was not considered necessary to give details of all the experiments.

In the 1930 experiments, five of the six soils had a very low nitrifying power for the 500 p.p.m. of ammonia nitrogen. The crop recovered more nitric nitrogen than ammonia nitrogen from two of these five soils, and recovered less nitric nitrogen from three soils. On an average, there was practically no difference between the availability of the nitric nitrogen and that of the ammonia nitrogen. The nitric nitrogen was more available than the ammonia nitrogen in the soil with the high nitrifying capacity.

Two of the same soils were used in both the 1930 and 1931 experiments, with different results. The corn took more nitrogen from nitrates than from ammonia from the Randall clay in 1930, and less in 1931. On the Amarillo fine sandy loam, corn took up more nitrogen from ammonia in 1930 than from nitrates, whereas the reverse occurred in 1931. This would indicate the error of the work to be at least the minimum differences found with these soils, namely 4 to 8 per cent of the nitrogen added.

In the 1931 experiments, the availability of the nitrate of soda was 3.8 to 9.2 per cent (average 6.9) greater than that of sulfate of ammonia with six of the seven soils low in nitrifying power. The Houston black clay subsoil was not included in this average. With it the difference was 43.2 per cent of the total amount of nitrogen added. The small differences are probably all in the limit of error and might be reversed another season, as was the case with the Randall clay and Amarillo fine sandy loam mentioned. In only the one soil was there a wide difference in availability between the nitrate and ammonia nitrogen. This was Houston black clay subsoil, a soil high in calcium carbonate. Possibly the calcium carbonate reacted with the ammonia to produce free ammonia, which was unfavorable to plant growth.

In the 1932 experiments, tobacco, sudan grass, oats, and wheat were grown. In two of the four soils low in nitrifying capacity, the nitric nitrogen was slightly less available than the ammonia nitrogen. In the other two, the nitric nitrogen was nearly 20 per cent more available than the ammonia nitrogen. One of these soils was a Houston black clay subsoil. Another sample of Houston black clay subsoil, from a different county, showed the same difference the previous year. This requires further study.

The nitric nitrogen was about 7 per cent (of the amount added) more avail-

TABLE 1

Relation of ammonia or nitric nitrogen recovered by crops to the nitrifying capacity of the soils

TYPE AND SOURCE OF SOILS	AMMONIA RECOVERED	NITRATE RECOVERED	GAIN OF NITRIC OVER AMMONIA NITROGEN	NITRIFYING CAPACITY	NITROGEN RECOVERED FROM COTTON-SEED MEAL	CROP
1930 experiments						
	per cent	*per cent*	*per cent*		*per cent*	
Randall clay, Potter county, 7–19″	87.4	93.6	+6.2	6	....	Corn
Amarillo fine sandy loam, Potter county, 7–19″	50.0	30.6	+19.4	6	....	Corn
Duval fine sandy loam, Frio county, 7–19″	81.7	73.6	−8.1	3	41.4	Corn
Hidalgo clay loam, Frio county, 7–19″	52.1	62.8	+10.6	100	34.5	Corn
Duval fine sandy loam, deep phase, Frio county, 7–19″	69.9	90.5	+20.6	2	47.4	Corn
Webb fine sandy loam, Frio county, 7–19″	71.2	68.0	−3.2	2	35.9	Corn
Total + (Except Hidalgo clay loam) (two soils)			+26.8	...	....	....
Total − (three soils)			−30.7	...	....	....
1931 experiments						
Randall clay, Potter county, 7–19″	64.9	61.1	−3.8	6	....	Corn
Amarillo fine sandy loam, Potter county, 7–19″	38.5	45.6	+7.1	6	....	Corn
Lake Charles clay, Galveston county	68.8	76.8	+8.0	0	....	Corn
Bowie fine sandy loam, Polk county, 18–24″	63.6	67.4	+3.8	0	....	Corn
Moscow fine sandy loam, Polk County, 7–10″	57.9	67.1	+9.2	0	30.2	Corn
Wilson clay, Collins county, 18–24″	76.8	83.3	+6.5	0	46.4	Corn
Trinity clay, Collins county, 7–24″	89.2	85.2	−4.2	100	55.5	Corn
Houston black clay, Collins county, 18–24″	33.4	77.6	+43.2	0	29.5	Corn
Total + (Except Houston black clay) (five soils)			+34.6	...	....	....
Total − (Except Trinity clay) (one soil)			−3.8	...	....	....
1932 experiments						
Sumter clay, Falls county, 0–7″	26.4	34.7	+8.3	87	14.8	Tobacco
Bell clay, Falls county, 0–7″	50.6	57.3	+6.7	93	25.7	Tobacco
Norfolk fine sand, Falls county, 0–7″	25.7	23.6	−2.1	0	10.6	Sudan
Susquehanna fine sandy loam, Falls county, 0–7″	19.5	39.1	+19.6	0	20.5	Sudan
Miles fine sand, shallow phase, Scurry county, 0–7″	33.0	30.4	−2.6	0	5.0	Oats
Houston black clay, Falls county, 24–40″+	21.5	40.9	+19.4	1	26.7	Wheat
Average of all soils (four) with high nitrifying capacity			+5.4	...	....	....
Average of all soils (sixteen) with low nitrifying capacity			+7.0	...	....	....

able than the ammonia nitrogen in the two soils used in 1932, having a high nitrifying capacity.

Summarizing all the results, we find that nitric nitrogen on an average was 5.4 per cent more available than ammonia nitrogen in the four soils having a high nitrifying capacity. The nitric nitrogen was 7.0 per cent more available than the ammonia nitrogen in the 16 soils having a low nitrifying capacity. These average differences are small and there is no indication of any regular relation between the nitrifying capacity of the soil and the availability of the nitric nitrogen and of ammonia nitrogen.

TABLE 2

Details of pot experiments on corn, 1930

SOIL, POT NUMBER, AND ADDITION	DRIED CROPS	ANALYSIS NITROGEN	NITROGEN	AVERAGE NITROGEN	NITROGEN RECOVERED	
	gm.	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*
Randall clay:						
1 DK	10.5	0.59	0.0620	0.0620	......	
2 DK, sulfate of ammonia	25.5	0.67	0.1709	......	......	....
3 DK, sulfate of ammonia	25.5	0.70	0.1785	0.1747	0.1127	87.4
4 DK, nitrate of soda	25.2	0.55	0.1386	......	......	....
5 DK, nitrate of soda	20.2	0.65	0.1313	0.1350	0.0730	93.6
Amarillo fine sandy loam:						
1 DK	20.5	0.48	0.0984	......	......	....
2 DK	29.9	0.48	0.1435	0.1310	......	....
3 DK, sulfate of ammonia	29.9	0.59	0.1764	......	......	....
4 DK, sulfate of ammonia	31.9	0.61	0.1946	0.1855	0.0645	50.0
5 DK, nitrate of soda	28.0	0.53	0.1484	......	......	....
6 DK, sulfate of soda	27.2	0.52	0.1414	0.1449	0.0239	30.6
Duval fine sandy loam:						
1 DK	13.5	0.51	0.0689	......	......	....
2 DK	13.4	0.51	0.0683	0.0686	......	....
3 DK, sulfate of ammonia	30.5	0.57	0.1739	......	......	....
4 DK, sulfate of ammonia	30.0	0.58	0.1740	0.1740	0.1054	81.7
5 DK, nitrate of soda	24.5	0.50	0.1225	......	......	....
6 DK, nitrate of soda	24.9	0.52	0.1295	0.1260	0.0574	73.6

It seems reasonable to conclude that the nitrogen of sulfate of ammonia is a little less available than that of nitrate of soda in soils of low nitrifying capacity as well as in soils of high nitrifying capacity. These experiments do not show whether nitric or ammonia nitrogen is utilized better by the plant after it is taken up, though no advantage of the one over the other may be seen in the vegetative growth of the plants in these experiments. It is also possible that other crops may show differences not shown by those used in these experiments. It is also possible that other soils may be found similar to the two Houston black clay subsoils, on which wide differences between the availability of the ammonia and nitric nitrogen were found.

Availability of nitrogen of cottonseed meal

In fourteen of the tests, cottonseed meal was tested in addition to the nitrate of soda and sulfate of ammonia, with the results shown in table 1. No particular relation can be seen between the nitrogen recovered from the cottonseed meal and the nitrifying capacity of the soil for ammonia. In 1930, the lowest recovery of nitrogen by the crops was with the soil having the highest nitrifying capacity; in 1931, the highest recovery occurred with a soil having a high nitrifying capacity. The recovery of nitrogen from cottonseed meal was about 45 to 65 per cent of that from nitrate of soda.

Nitrification capacity after cropping

The nitrifying capacity was tested on eight of the soils after they had been cropped. The addition of the fertilizer in the pot experiments and the cropping increased the nitrifying capacity of three of eight of the soils, but did not affect that of the other five. No relation could be seen between the effect of the cropping on nitrification and the relative availability of the nitrogen of sulfate of ammonia and of nitrate of soda.

Relation to causes of the low nitrifying capacity

Tests were made to ascertain whether the low nitrifying capacity of the soils used was due to deficiency of carbonate of lime or of nitrifying bacteria or of both (2). In some soils the low nitrifying capacity was due to deficiency of carbonate of lime, and in other soils it was due to deficiency in nitrifying organisms. Four of the six soils on which the assimilation of nitrogen of nitrate of soda was higher than that of sulfate of ammonia in 1930 were found to be deficient in nitrifying bacteria.

SUMMARY

Nitrate nitrogen was, on an average, 5.4 per cent more available than ammonia nitrogen in four soils having a high nitrifying capacity. Nitric nitrogen was 7 per cent more available than ammonia nitrogen in 16 soils having a low nitrifying capacity. The difference between the two groups is small and within the experimental error. There is no indication of a regular relation between the availability of nitric nitrogen and of ammonia nitrogen and the nitrifying capacity of the soil. On two samples of Houston black clay, subsoil, the nitrate nitrogen was decidedly more available than the ammonia nitrogen.

The availability of nitrogen of cottonseed meal was lower than that of either nitrate of soda or sulfate of ammonia. No relation was found between the availability of nitrogen in cottonseed meal and the nitrifying capacity of the soils.

The nitrifying capacities of five soils were practically the same after cropping as before cropping in the pot experiments. The nitrifying capacity of three of the soils appeared to be increased by the cropping and addition of fertilizer.

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THE DISTILLATION METHOD FOR DETERMINING THE COMBINED WATER AND ORGANIC MATTER IN SOILS[1]

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In a previous communication (2) the distillation method was presented as a possible means of determining accurately the combined water and organic matter in soils. The combined water is defined as that water which remains in the soil after it has been dried for 24 hours at a temperature of 110°C. It may be water of crystallization, constitution, or adsorption, or combinations of these. The principle of the method consisted of placing a definite amount of air-dry soil in a bomb, heating it at a high temperature, and condensing and catching the water distilled over. On a separate sample of the same soil were determined the hygroscopic moisture content and its loss on ignition. By subtracting the hygroscopic moisture from the total water distilled over, the combined water was obtained, and by subtracting the combined water from the loss on ignition of the oven-dry soil, the organic matter was obtained.

The method and results as presented in the first report were preliminary and tentative and it was stated that the investigation of the whole subject was being continued with special efforts being made to improve the technique and apparatus. It is the object of this paper to report on the method as finally perfected and the results it yields on the determination of combined water and organic matter in soils.

METHOD AND PROCEDURE

The original apparatus used for determining the amount of combined water in soils has been considerably modified and improved. The one finally adopted and used in the present investigation is shown in figure 1. It consists of a 250-cc. bomb made of iron pipe. The cap is screwed on. In a hole in the center of the cap is brazed a short ½-inch pipe and to this pipe is brazed a short ⅛-inch copper tube. The bomb is connected to a Liebig condenser by means of a rubber stopper at the end of the copper tube. The bomb is then placed in the electric muffle and the latter closed with an improvised asbestos door, made of ordinary asbestos sheet. The door of the muffle itself is let down in order to facilitate the placing of the bomb in the muffle. The condenser jacket can be either connected to a faucet with running water or kept filled with cold water, but preferably the former. The water distilled over is caught in a special very narrow cylinder graduated to 0.1 cc.

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The final procedure for determining the combined water consists of first preparing the soil by passing it through a 2-mm. sieve, then testing it for carbonates. If carbonates are present they are destroyed with hydrochloric acid and then the soil is thoroughly washed, dried, and screened again. Soils that have no carbonates are not treated with the acid. Exactly 100 gm. of the air-dry soil, in the case of mineral soils, and 20 gm. in the case of mucks and peats, are then placed in the bomb and about 30 gm. in a crucible. The latter sample is used to determine the hygroscopic moisture and the ignition loss of

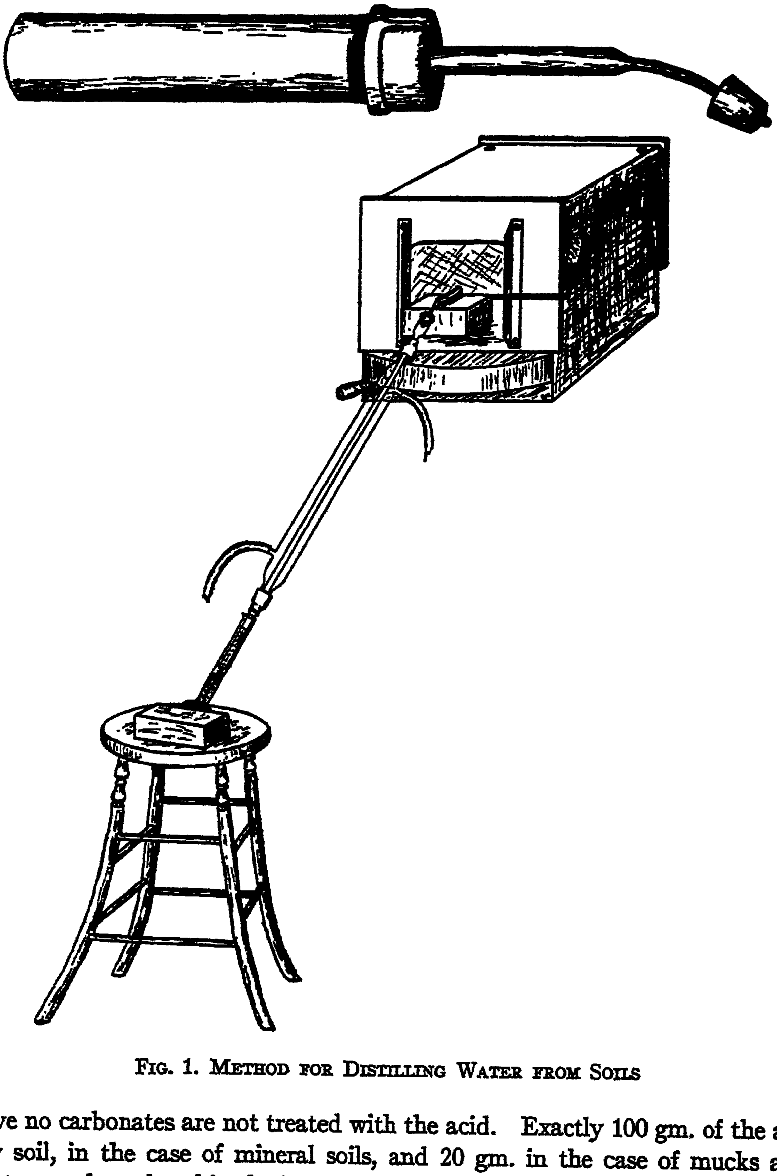

FIG. 1. METHOD FOR DISTILLING WATER FROM SOILS

the soil. The lid is screwed on the bomb and the joints are covered with a layer of asbestos cement to make sure that the bomb is air-tight. The cement after it is put on is gently tapped or pressed against the joints with the finger. To prevent the cement from sticking on the finger, the latter is smeared with soil dust. To take off the cement after it is dried on the bomb, the cap of the bomb is hit with a hammer and the cement breaks off readily. The process of putting on and taking off this asbestos cement is very simple and does not take more than 1 minute. The bomb is now placed in the electric muffle, the cylinder is connected to the end of the condenser tube, and the jacket is connected to the faucet. The current is turned on and regulated to produce the maximum temperature at the speed desired. Soils that contain no organic matter or only a moderate amount, are heated very rapidly or placed immediately at a temperature of 900°C. Mucks and peats and mineral soils containing very large amounts of organic matter are heated first at 330°C. and allowed to remain at this temperature until they give up most of their combined water, and then their temperature is raised to about 500°C. or 900°C., depending upon the clay content. The water distills very rapidly and is caught in the graduated cylinder. When the distillation is completed, the bomb is disconnected from the condenser. The disconnecting is done by first turning off the water at the faucet and then taking hold of the cylinder and the tube of the condenser with one hand, and with the other pushing out the rubber stopper from the mouth of the condenser tube. The condenser is then held up straight so that any drops of water that might remain in the tube will drain into the cylinder. The cylinder is set on the table and the water in the jacket is drained off by disconnecting the rubber tubes. The condenser tube is again connected to the cylinder containing the distilled water and the tube is carefully rinsed with carbon tetrachloride. The water rises to the top of the carbon tetrachloride and its exact volume is read and recorded. The condenser tube is then cleaned thoroughly with alcohol and set aside to dry. The hot bomb in the muffle is taken out and replaced by another one containing different soil, and the whole process described in the foregoing is repeated.

It was found that the mineral soils, and especially the clays, should be heated to 900°C. in order that all their combined water may be expelled from them. If the soils are placed directly in the temperature of 900°C., they seem to lose all their combined water in less than 30 minutes. In fact, soils that are low in colloidal mineral content such as Marion silt loam, surface, give up all their combined water in about 15 minutes. This point was repeatedly proved by extending the duration of the distillation to over an hour without any additional water being distilled. When the water is being expelled from the soil, the bulb or upper part of the inner condenser tube is hot from the steam and can hardly be touched by the hand, but when all the combined water is expelled from the soil this bulb becomes cold, indicating that no more water is being expelled, and can be held by the hand. This point can be used as an excellent aid in determining when distillation is completed.

In the case of the mucks and peats it was found that they would give up nearly all of their combined water by being heated to a temperature of only about 330°C. Only a small amount of additional water would be given up at higher temperatures. Mucks and peats, therefore, were first heated to about 330°C. until they gave up most of their water and were then finally heated to about 500°C.

Organic matter when placed directly in a temperature of 900°C. gives off smoke in a rush, but when placed at lower temperature or heated gradually to the high temperature practically no smoke is given off. The best way to heat mucks and peats, and mineral soils containing extremely large amounts (above 10 per cent) of organic matter, is to place them first in a temperature of about 330°C., and then raise the temperature to 500° or 900°C. Soils containing moderate amounts of organic matter can be placed directly at the temperature of 900°C. without producing very much smoke.

In nearly all of the soils some volatile solids are also distilled over in addition to the water. These are mainly tar arising from the organic matter, although in some soils the material seems to be more like sulfur. Soils that contain less than 10 per cent organic matter produce only traces or very small amounts of tar, but mucks and peats produce considerable amounts of this material. The tar tends to condense in mass and stick on the wall of the condenser tube while the water expelled and falling into the cylinder is rather clear. Since in the case of the mineral soils the amount of this tar is very small, it is all washed, by the carbon tetrachloride, into the cylinder. The water rises to the top of the carbon tetrachloride and is clear except that sometimes there may be a very thin layer of coagulated organic matter between the carbon tetrachloride and water columns, but this causes no difficulty or error in the readings. Since the amount of the tar in the case of the mucks and peat is large, care is taken to rinse the condenser tube with carbon tetrachloride without washing all the tar mass into the cylinder and thus make it somewhat difficult to read the column of water accurately. As the tar has a tendency to stick to the walls the tube can be easily rinsed without dislodging the tar mass. When these precautions are taken it is seldom that the distilled water in the cylinder is not clear enough to be read accurately.

It was found that when the mucks and peats are heated and especially in low temperatures or when the temperature is raised gradually, the water is expelled first before the tar begins to come out. This fact together with the rinsing operation with carbon tetrachloride eliminates the possibility of any appreciable amount of water being held back by the tar left in the condenser tube.

On account of the volatile solids condensing and sticking in the condenser tube, and in order that all the water distilled may be accurately delivered into the cylinder, the condenser tube is thoroughly cleaned with alcohol at the end of every experiment or run. The alcohol cleans the tar out very readily. The condenser tube is allowed to dry out before it is used again.

Before the method was employed to obtain final experimental results it was tested to determine the degree of accuracy. The procedure consisted of adding definite amounts of water to quartz sand and to ignited soils having no hygroscopic or combined water, and then trying to recover the water added. The results obtained are shown in table 1.

It is seen that the quantity of water recovered in each case agrees quite satisfactorily with the quantity of water added. This test together with the excellent agreement between combined water and loss on ignition in soils containing no organic matter, as revealed in table 2, indicates that the method as finally perfected gives very reliable results.

Since in order to obtain the combined water the hygroscopic moisture has to be subtracted from the total water distilled over, and since to obtain the organic matter the combined water has to be subtracted from the loss on ignition, it is essential that both the hygroscopic water and ignition loss be accurately determined. The hygroscopic moisture is determined by drying the soils in an electric oven at a temperature of 110°C. for 24 hours. For loss on ignition the soils are heated in a temperature of 900°C. for about 5 hours. It is well always to examine and see that combustion is complete.

TABLE 1

Tests of the degree of accuracy of the distillation method

SOILS	WATER ADDED	WATER RECOVERED
	cc.	*cc.*
Quartz sand, dried	10.00	9.90
Decatur clay, ignited	10 00	10.00
Hagerstown clay loam, ignited	10.00	10.00
Irredell loam, ignited	10.00	9.90
Susquehanna clay, ignited	10.00	9.90
McKenzie clay, ignited	10.00	10.00

As already indicated, the term "combined water" is a general term, not a specific one. It is employed for convenience and is intended to designate all that water in soils which is not driven off at the temperature of 110°C. for 24 hours. This limit of temperature is chosen because it is the temperature which is generally used to dry soils for scientific investigations and chemical analysis. From a strictly chemical viewpoint, the water that is distilled over at the high temperatures is not all water of combination or of constitution, but a portion of it is adsorbed water which can be driven off only at very high temperatures. But since it is difficult to segregate definitely this adsorbed water from the true water of combination or constitution, it is all designated at present as combined water.

The method, as can be seen from the foregoing description, is very simple of operation and requires no special technique. It is also rapid of execution.

TABLE 2

Combined water as obtained by the distillation method, and organic matter determined indirectly by calculating the difference between combined water and ignition loss

Soils 1–33 contained no organic matter or carbonates

SOILS	DEPTH	$CaCO_3$	HYGROSCOPIC WATER AT 110°C.	LOSS ON IGNITION FROM SOILS DRIED AT 110°C.	COMBINED WATER DISTILLED OVER FROM SOILS DRIED AT 110°C.	ORGANIC MATTER CORRECTED FOR CARBONATES AND COMBINED WATER
	inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Silica gel	.....	0	0.66	4.39	4.37	.....
Bentonite	.....	0	12.22	5.07	4.88	.....
Fullers earth	.....	0	8.37	9.65	9.53	.....
Hagerstown clay loam	70–80	0	4.29	7.85	8.00	.....
Hagerstown clay loam	50–70	0	3.75	7.46	7.57	.....
Hagerstown clay loam	30–50	0	2.83	7.49	7.28	.....
Hagerstown clay loam	20–30	0	3.23	7.85	7.97	.....
Hagerstown clay loam	12–20	0	2.81	8.24	8.09	.....
Davidson clay loam	24–30	0	2.07	8.34	8.46	.....
Irredell loam	12–16	0	5.45	8.57	8.31	.....
Greenville fine sandy loam	12–18	0	1.18	4.66	4.80	.....
Fallbrook sandy loam	14–27	0	1.97	3.08	2.93	.....
Bladen loam	12–18	0	3.08	5.46	5.30	.....
Antioch clay loam	40–60	0	3.77	3.07	2.88	.....
Bladeley loam	8–15	0	1.24	2.96	2.81	.....
Decatur clay	0–6	0	1.20	4.14	4.04	.....
Decatur clay	6–12	0	2.06	5.45	5.50	.....
Decatur clay loam	30	0	1.97	5.71	5.78	.....
Decatur clay	84	0	2.24	5.82	5.74	.....
Clay	C horizon	0	3.66	3.88	3.61	.....
Colbert clay	48	0	6.38	6.86	7.02	.....
Bladden loam	12–18	0	2.16	5.46	5.31	.....
Susquehanna clay	2–6	0	3.92	6.31	6.48	.....
Susquehanna clay	16–20	0	3.83	4.44	4.49	.....
Susquehanna clay	24	0	3.65	4.69	4.55	.....
Susquehanna	48	0	5.79	5.93	6.11	.....
Ontonagon clay	B horizon	0	2.08	2.90	3.03	.....
Ontonagon clay	C horizon	28.4*	2.39	4.09	3.95	.....
Fulton clay	C horizon	15.80*	2.36	3.78	4.00	.....
Marengo clay	4–30	0	8.76	5.56	5.38	.....
Lufkin clay	10–16	0	10.04	6.19	5.90	.....
Nipe clay	.....	0	2.90	11.01	10.75	.....
San Joaquin sandy loam	0–14	0	1.08	2.35	2.19	.....
Marion silt loam	Surface	0	1.85	5.38	2.94	2.44
Lindley silt loam	Surface	0	1.99	4.31	2.60	1.71
Putman silt loam	Surface	0	2.33	6.81	2.99	3.82
Clinton silt loam	Surface	0	1.72	4.88	2.55	2.33
Grundy silt loam	Surface	0	2.74	6.66	4.10	2.56
Clarion silt loam	Surface	0	2.32	7.30	3.21	4.09
Bumer silty clay	Surface	0	3.91	7.22	3.79	3.43
Colby silt loam	Surface	0	2.43	6.64	3.12	3.52

TABLE 2—*Continued*

SOILS	DEPTH	$CaCO_3$	HYGROSCOPIC WATER AT 110°C.	LOSS ON IGNITION FROM SOILS DRIED AT 110°C.	COMBINED WATER DISTILLED OVER FROM SOILS DRIED AT 110°C.	ORGANIC MATTER CORRECTED FOR CARBONATES AND COMBINED WATER
	inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Colby silt loam..........	Subsoil	0	1.83	3.63	2.55	1.08
Altmont adobe..........	0–20	0	4.86	7.10	4.82	2.28
Fargo clay loam.........	Surface	0	7.82	12.00	6.18	5.82
Fargo clay loam.........	6	0	5.38	11.70	4 35	7.35
Fargo clay loam.........	6–16	0	5.23	8.44	4.06	4.38
Haldemond clay..........	Surface	0	3.34	9.20	4.11	5.09
Welland clay............	Subsoil	0	3.05	5.13	4.01	1.12
Napanee silt loam........	Surface	0	2.58	7.63	3.37	4.26
Yolo silt loam...........	1–18	0	4.24	9.92	5.27	4.65
Miami silt loam..........	Surface	0	2.34	3.43	2.16	1.27
Clyde clay..............	Surface	0	6.77	20.55	6.96	13.59
Lake Charles clay........	Surface	0	4.48	5.61	3.27	2.34
Webster silty clay loam...	Surface	0	6.08	13.16	4.32	8.84
Clay...................	B horizon	0	5.09	6.70	5.44	1.26
Clay...................	A horizon	0	1.02	2.78	1.78	1.00
Bladen loam.............	0–8	0	1.21	5.70	2.30	3.40
Davidson clay loam......	0–6	0	2.12	9.95	6.56	3.39
Montezuma clay loam....	0–27	0	4.16	4.96	3.36	1.60
McKenzie clay...........	0–1	0	5.15	6.74	3.92	2.82
Hagerstown clay loam....	0–1	0	1.72	8.67	3.87	4.80
Hagerstown clay loam....	1–4	0	0.876	2.92	2.65	0.27
Hagerstown clay loam....	3–6	0	1.40	3.67	3.10	0.57
Hagerstown clay loam....	6–12	0	2.36	6.29	5.74	0.55
Black clay adobe.........	.....	0	5.43	8.59	7.79	0.80
Irredell loam...........	0–5	0	1.57	3.89	2.75	1.14
Sacramento clay.........	Surface	0	5.50	8.46	5.82	2.64
Kirwin fine sandy loam...	8–12	0	4.08	7.37	6.83	0.54
Lamoure silty clay loam...	Surface	0	3.13	13.01	4.51	8.50
Lufkin clay.............	0–3	0	8.69	11.88	5.98	5.90
McKenzie clay...........	A horizon	0	7.33	9.72	5.29	4.43
Parsons silt loam.........	B horizon	0	5.08	6.55	4.83	1.72
Colbert clay............	0–6	0	3.32	5.66	5.16	0.50
Stockton clay adobe......	0–16	0	8.21	7.11	6.71	0.40
Marshall silt loam........	0–12	0	5.34	5.56	2.83	2.73
Lufkin clay.............	0–7	0	8.96	5.86	5.44	0.42
LaFra clay.............	0–4	0	5.31	8.02	5.24	2.78
Summit silt loam.........	28–40	0	4.49	4.68	4.12	0.56
Poncena clay............	0–7	0	2.71	12.10	7.59	4.51
Miles fine sandy loam.....	Subsoil	0	2.53	3.64	3.32	0.32
Colbert clay............	8	0	5.66	7.68	7.05	0.63
Vista sandy loam........	24–43	0	1.07	2.06	1.56	0.50
Soil compost............	.....	0	8.16	16.00	1.84	14.11
Soil compost............	.....	0	4.66	18 30	3.32	14.98
Soil compost............	.....	0	7.92	19.35	2.83	16.52

TABLE 2—*Concluded*

SOILS	DEPTH	$CaCO_3$	HYGROSCOPIC WATER AT 110°C.	LOSS ON IGNITION FROM SOILS DRIED AT 110°C.	COMBINED WATER DISTILLED OVER FROM SOILS DRIED AT 110°C.	ORGANIC MATTER CORRECTED FOR CARBONATES AND COMBINED WATER
	inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Muck 1	.....	0	11.67	68.75	12.89	55.86
Muck 2	.....	0	8.52	79.35	14.78	64.57
Muck 3	.....	0	12.64	71.70	13.26	58.44
Muck 4	.....	0	10.78	81.50	13.56	67.94
Peat 1	.....	0	13.77	86.70	14.66	72.04
Peat 2	.....	0	7.93	90.60	15.87	74.73

* Carbonates destroyed by hydrochloric acid.

Since the distillation of any soil can be completed in from ½ to 1 hour, more than 12 soils can be easily distilled in 1 day. With the time required to determine the hygroscopic moisture and ignition loss, the complete determination of combined water and organic matter can be easily made on more than 12 soils in 2 days if only one drying oven and one muffle furnace are used.

EXPERIMENTAL RESULTS

In table 2 are presented the experimental results as obtained by the distillation method on combined water and organic matter in soils. Ninety-one soils and artificial materials were examined. A special effort was made to investigate a large number of representative types of soil of different chemical composition, clay, and organic matter content, and of different depths.

The results in table 2 show that both the combined water and organic matter vary greatly in the different soils.

The data on combined water and ignition loss of the first 33 soils present the real test as to the soundness and accuracy of the distillation method for determined combined water in soils. These first 33 soils and artificial materials, as far as could be ascertained, contain no organic matter or carbonates. Provided that the loss on ignition is due to combined water, as it certainly must be in silica gel and bentonite, then the combined water as obtained by the distillation method should equal the loss on ignition. That is exactly what takes place. It will be seen that the figures for combined water and ignition loss for all of these soils and artificial materials are remarkably close. This close agreement is significant and should be considered as affording a real test of the method as to its soundness and accuracy for determining the combined water in soils.

When the soils containing organic matter are examined, however, it is seen that the figures for combined water and ignition loss are far from being in close agreement. This disagreement is of course due to the organic matter which is present in the soils as revealed in the last column of the table.

The greatest uncertainty in the method is whether the water that is distilled over from the soil organic matter itself can really be classed as combined water, the same as that obtained from inorganic soil material. On account of preconceived ideas in regard to the water in inorganic hydrates, and in view of the general nature of the soil organic matter, it is probably difficult to consider the water yielded by the soil organic matter on destructive distillation as combined water and to be the same as that in inorganic soil material. On the other hand there is no absolute proof that it is not water of combination, and any distinction made at present is probably an artificial one. Mellor (4) in discussing the subject of inorganic hydrates states:

> The mode of writing the formulae—$CuSO_4 \cdot 5H_2O$; $Na_2SO_4 \cdot 10H_2O$—and the ease with which the hydrates dissociate into water, etc. might give rise to the idea that the water molecules exist in the hydrate ready made. There is, however, no evidence how the elements of water are combined in the hydrate. We know very little beyond the simple fact that water is a product of the dissociation of the hydrates.

He says further (5):

> The very definite amount of water which is contained in many salts which crystallize from aqueous solutions—e.g., sodium carbonate, $Na_2CO_3 \cdot 10H_2O$, etc.—is called water of crystallization and several other synonyms have been employed. It is often stated that the molecules of water in compounds containing water of crystallization belong to the molecular structure or else exist in them entirely among the other molecules, and belong only to the crystalline structure. On the other hand, the molecule of water of constitution is not supposed to exist as such but to be formed when the mineral is decomposed, owing to the union of the contained hydrogen with the oxygen or hydroxyl groups contained in the molecule.

It is to be specifically noted in the last sentence of the foregoing that the water of constitution is not regarded as existing as water in the mineral but that it is formed by the union of hydrogen and oxygen contained in the molecule of the mineral when the latter is decomposed.

Alexander Smith (8) in discussing the subject of inorganic hydrates also, states:

> The ease with which some of the hydrates decompose suggested the idea that they contain water as a discrete substance. There is no more justification for this idea, however, than for the notion that carbonates contain ready-made carbon dioxide. The hydrates contain the elements of water just as sugar and alcohol do and there is no evidence that they contain water in any other sense than that in which the phrase might be used to these organic bodies.

Coblentz (3) studied the infra-red absorption spectra of a large number of minerals with the purpose in view of distinguishing the form in which they contained their water. He found that absorption spectra of selenite, opal, and the zeolites are identical with those characteristic of free liquid water, and therefore it is inferred that the water in these minerals remains intact as a group of molecules. On the other hand, in brucite $Mg(OH)_2$; diaspore, $AlO(OH)$; bauxite, $Al_2O(OH)_4$; göthite, $FeO(OH)$; muscovite mica, $H_2KAl_3(SiO_4)_3$; tremolite, $CaMg_3(SiO_3)_4$, the water is not present in solid solution or as absorbed water, but is rather constitutional, being an integral part of the molecule. Many of these minerals show a clear absorption band characteristic of the OH group in alcohol.

If these and other authorities are willing to consider the inorganic hydrates like $CuSO_4 \cdot 5H_2O$ and $Na_2CO_3 \cdot 10H_2O$ as containing the elements of water the same as sugar and alcohol do, then certainly the soil organic matter contains the elements of water the same as the inorganic soil hydrates do, and the water can be classed in both cases as combined water. Furthermore, if the term "water of constitution" as defined in the foregoing can be applied to minerals and salts, then there can be no distinction made between the combined water in inorganic and organic soil material, because the chief basis for a differentiation is thereby eliminated. For instance, the greatest objection that could be offered against calling the water in organic matter combined water is that it might not exist as water but that water is formed upon the decomposition of the organic matter. Now if it is admitted that water is formed when the minerals decompose and this water is called water of constitution and combined water, then the water that might be formed when the organic matter is decomposed by heating is also water of constitution and combined water, and consequently there is no difference between it and that of the inorganic soil.

There are some additional evidences which go to support the idea that the water yielded by the organic matter is combined water the same as in the inorganic soil material or as in kaolin. For instance, soil organic matter is colloidal in nature, and its chemical and physical behavior, such as base exchange, amphoteric reaction, and heat of wetting, is very much the same as in inorganic soil material. Again, in order to expell all the water they contain, both the organic and inorganic soil materials have to be heated to a temperature which entirely destroys their original state and neither one will revert to it again by the addition of water.

It might be argued that the water yielded by the organic matter is formed or manufactured by oxidation in the sense that during heating the hydrogen of the organic matter combines with oxygen of the atmosphere to form water, in which event the water would not be part of the organic matter. The formation of water by oxidation in this sense is very improbable for two reasons. In the first place, the heating takes place in a closed bomb. In the second place, any free oxygen gas that is contained in the bomb and in the organic matter is largely expelled by the rise in temperature before decomposition of the organic matter begins. It is difficult to see, therefore, how there would be enough free oxygen in the bomb to combine with the hydrogen of the organic matter to form water.

From all evidences in our present knowledge, it appears, therefore, that there is no proved justification of making a distinction between the combined water in the inorganic and organic soil materials. The definition, therefore, that "combined water may be water of adsorption, crystallization, or constitution, or combinations of these" applies to organic soil material as well as to the inorganic.

In the event, however, that the water yielded by the organic matter does not comply with the definition of combined water as given in the foregoing and that it is entirely different from the water yielded by the inorganic soil material, then there is a small error introduced in the distillation method for calculating the organic matter content of soils. This error is estimated to be about 1 per cent low in soils containing 10 per cent organic matter and proportionally higher or lower depending upon the organic matter content present. In other words, if the distillation method shows a soil to contain 10 per cent organic matter, then the correct amount of organic matter is about 11 per cent. In estimating this error two things are taken into consideration: first, the amount of water that mucks and peats yielded; and second, the amount of adsorbed moisture that the organic matter might contain which is not expelled at the temperature of 110°C., but is driven off at the higher temperatures. In regard to the latter point the work of Nelson and Hulett (6) shows that the amount of moisture that would be expelled from wheat flour, corn meal, cellulose, protein, and other colloidal organic substances when heated to the temperature of 218°C. under vacuum after they had already been dried at 100°C. was about 2 per cent in many cases. Undoubtedly in the case of mucks which have been decomposed and possess a greater surface, the amount of this adsorbed water is much greater than 2 per cent, as some unpublished data show.

Because it would be of great interest to know how the distillation method compares with the present standard methods for determining organic matter in soils, it is planned to investigate thoroughly the distillation method with the combustion method, the hydrogen peroxide method of W. O. Robinson, and the Rather method.

Already a preliminery comparison has been made between the distillation method and the combustion method.[2] Although the combustion method is not an absolute method, since it makes use of an empirical factor for converting the carbon dioxide to organic matter, and this empirical factor may be in error (7) as much as 75 per cent, nevertheless the method seems to be approximately correct for the average soil and it is of interest to see how it checks with the distillation method. The comparative results obtained on nine unknown soils by the two methods are shown in table 3. It is seen that the two methods agree fairly closely in six soils out of nine, but they disagree rather appreciably in three. The greatest disagreement is in the Hagerstown silty clay loam. Although no definite explanation can be given at present for the disagreement in the three soils, i.e. Poncena clay, Webster silty clay loam, and Hagerstown silty clay loam, it seems probable that in the case of the Hagerstown silty clay loam the combustion method gives too high a figure for organic matter. In the first place, this soil is very light in color and does not give evidence that it

[2] In this preliminary comparison the writer is much indebted to Dr. L. D. Baver of the University of Missouri, who kindly made the combustion determinations.

contains much organic matter. In the second place, its nitrogen content is only 0.1727 per cent as compared to 0.3097 per cent nitrogen in the case of Marion silt loam, whose organic matter content is less than half of that of Hagerstown silty clay loam. It seems very probable, therefore, that at least in the case of the Hagerstown silty clay loam, the empirical factor fails to give the correct organic matter content.

With the exception of the three soils, therefore, the combustion method agrees fairly well with the distillation method, especially since the critical comparison made by the U. S. Bureau of Chemistry and Soils (1) on the combustion, hydrogen peroxide, and the Rather methods does not show much better agreement than that shown by the distillation and combustion methods.

TABLE 3

Comparison between the combustion method and the distillation method for determining organic matter in soils

SOILS	DEPTH	ORGANIC MATTER	
		Combustion method $CO_2 \times 0.471$ (Baver)	Distillation method
	inches	*per cent*	*per cent*
Marshall silt loam	0–12	3.57	2.73
Lufkin clay	0– 7	1.03	0.42
Summit silt loam	28–40	0.96	0.56
Poncena clay	0– 7	6.36	4.51
LaFra clay	0– 4	3.05	2.78
Clyde clay	Surface	14.46	13.59
Webster silty clay loam	Surface	10.30	8.84
Hagerstown silty clay loam	0–1	7.16	4.80
Marion silt loam	Surface	3.32	2.44

On the other hand, it must be realized that if the water yielded by the organic matter is to be regarded as combined water the same as that in the inorganic soils, then the distillation method will not agree exactly with these standard methods, since they do not take into consideration this combined water in their calculation of the organic matter. The disagreement, however, will be very small except in mucks and peats.

COMPARISON BETWEEN COMBINED WATER AND CLAY CONTENT OF SOILS THAT CONTAIN NO ORGANIC MATTER

Since the results in table 2 tend to show that next to muck and peats, clay soils contain the largest amount of combined water, a study was made to determine how close the relationship is between clay content and combined water. In order to eliminate the influence of organic matter, only soils that contain no organic matter were chosen for the comparison.

The data in table 4 show emphatically that there is no close and consistent relationship between clay content and combined water in the various soils. This lack of relationship is in line with expectations, since not only clay content but chemical composition should influence the combined water in soils.

TABLE 4

Comparison between combined water and clay content of soils

SOILS	DEPTH	COMBINED WATER	CLAY 0.005–0 MM.	FINE CLAY 0.002–0 MM.	RATIO: CLAY / COMBINED WATER	RATIO: FINE CLAY / COMBINED WATER
	inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Hagerstown clay loam......	12–20	8.09	84.4	82.3	10.45	10.18
Irredell loam..............	12–16	8.03	74.1	70.4	9.23	8.77
Susquehanna clay..........	16–20	4.49	41.0	39.5	9.13	8.80
Susquehanna clay..........	48	6.11	64.8	63.4	10.60	10.38
Davidson clay loam.........	24–30	8.46	71.2	69.1	8.42	8.17
Decatur clay...............	6–12	5.50	42.3	40.7	7.69	7.35
Greenville fine sandy loam...	12–18	4.80	39.6	37.5	8.25	7.82
Bladen loam................	12–18	5.30	54.0	48.8	10.19	9.21
Antioch clay loam..........	40–60	2.88	34.8	32.4	12.08	11.25
Lufkin clay.................	10–16	5.90	90.2	85.2	15.30	14.44
Marengo clay..............	4–30	5.38	96.0	92.8	17.85	17.25
Colbert clay................	48	7.02	74.8	73.3	10.66	10.45

SUMMARY

Further work is presented on the distillation method for determining combined water and organic matter in soils.

The original apparatus and technique have been modified and improved.

It is indicated that the method is accurate in determining the amount of water that is distilled over.

The method also appears to be accurate in determining the organic matter content in soils. The only uncertain factor in the determination of the organic matter is whether the water yielded by the organic matter upon destructive distillation is combined water, the same as in the inorganic soil material.

Evidences are deduced to show that the water in the organic matter can be regarded as combined water the same as in the inorganic soil material.

The technique and procedure for determining combined water and organic matter are simple and rapid.

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BOOK REVIEWS

Der Bau des Ackerbodens. Gemeinverständlicher Abriss der auf den Ackerboden angewandten Kolloidforschung. By Prof. PAUL EHRENBERG. Theodor Steinkopf, Dresden. Pp. 96.

The author of "Die Bodenkolloide" has produced a new work on the application of the principles of colloid chemistry to soil science and practice. The book represents a popularized and abridged edition of "Die Bodenkolloide," unburdened by the extensive bibliography of the preceding work. This has enabled the author to present the subject in a more condensed and readable form and to make the book comprehensible to the educated practical farmer as well as to the student and soil scientist in general. The author has achieved considerable success in illustrating the more difficult abstractions in colloid chemistry by the use of analogous common, everyday phenomena.

The book is divided into three parts as follows:

A. The colloids of the soil.
B. The colloidal phenomena in the soil.
C. Soil structure (Der Bau des Bodens).
  I. The destruction (Abbau) of the soil: (1) In nature, (2) By man.
  II. The upbuilding (Aufbau) of the soil: (1) In nature, (2) By man.

The frequent use of the term "Bodengare" is very interesting and instructive. The word, which is difficult to translate, recalls Hilgard's "crumb structure" of the soil, and means good tilth and everything that makes the soil favorable for crop production: The soil is "gar" (done) when it is in its best form to receive the seed. Bodengare is the aim of all cultural practice.

The dominating importance of colloid chemistry to soil science and practice has come to be more and more recognized during later years by the specially trained group of soil investigators. But the large body of teachers and educated practical agriculturalists have not yet acquired a sufficient knowledge of this subject. It is in particular to this group of readers that the new book will be welcome but it will also prove useful to the soil student and investigator in general. The book is worthy of the highest recommendations.

SANTE MATTSON.

Vorlesungen über Boden-Mikrobiologie. By AUGUST RIPPEL. Julius Springer, Berlin, 1933. Pp. viii + 161.

This volume supplements the author's "Vorlesungen über theoretische Mikrobiologie," which appeared several years ago. The 26 chapters deal, respectively, with the significance of soil microbiology, the methodology of soil microbiology, the distribution of microorganisms in the soil, the carbon cycle (four chapters), the cycle of nitrogen (ten chapters), the cycle of sulphur and iron, the formation and decomposition of humus materials (three chapters), the influence of microbiological processes of soils (two chapters), the use of microbiological methods for the measuring of soil fertility, the microbiology of water, and the conservation of organic substances.

The book contains the substance of the author's lectures at Göttingen, and deals also with laboratory exercises. It was evidently the author's intention to make the volume useful for landowners, biologists, pharmacists, and chemists.

Untersuchungen über die Mikrobiologie des Waldbodens. By D. FEHER. Julius Springer, Berlin, 1933. Pp. vi + 272, figs. 76, tables 64.

The author notes in the introduction that the data presented in his book are the result of a decade of carefully planned studies at the Botanical Institute of the Royal Hungarian College for Mining and Forestry Engineers at Sopron, Hungary. In preparing the book for publication, he had the collaboration of R. Bokor and L. Varga.

As a guiding principle, the author and his collaborators have stressed the study of the relations in the dynamic sense of the physiological and ecological phenomena which dominate the physiological functions of forests. It is proposed by the author to supplement the present work by other publications. Aside from the introduction, the book is made up of 10 chapters. These deal with experimental methods; bacteria of forest soils; the microbiological factor as related to carbon dioxide formation in soils; microbiological study of the nitrogen cycle in forest soils; seasonal variations in the biological transformations in forest soils; microbiological properties of sandy soils; microbiological fungi of forest soils; studies of regional distribution of algae in European forest soils; protozoa in forest soils, and the microbiology of "Szik" soils with special reference to their improvement. The book is well illustrated and includes a fine list of references.

Quantitative Bacteriology. By H. O. HALVORSON AND N. R. ZIEGLER. Burgess Publishing Company, Minneapolis, Minn., 1933. Pp. 64.

In preparing this mimeographed volume, the authors had in mind the making available to other bacteriologists of certain quantitative data for use by the students and investigators. They point out that:

> In biological work numerous problems arise that require the solution of complex algebraic equations. For example, in determining the most probable bacterial population by the dilution method, and the limits of accuracy of the data obtained, involved equations must be worked out. If the biologist wishes to determine the extent of infestation of insects with certain viruses or bacteria, the calculation of the percentage of infestation from experimental data also involves the solution of complex equations. The solution of these and other problems may be facilitated by a series of tables of standard functions. Some of these tables may be found in handbooks or original publications, but in no instance have they been collected

> for the convenience of the biologist. It is the object of this book to supply a much-needed collection of these tables.

The authors have placed their colleagues under debt to them for having performed this task.

Untersuchungen über das Kalkbedürfnis der Böden durch Laboratoriumsmethoden und Düngungsversuche. Compiled and edited by O. LEMMERMANN with the collaboration of L. FRESENIUS. Verlag Chemie, G. m. b. H., Berlin, 1933. Pp. 463.

The author is one of the leading authorities in the field of soil science. He has been able to gather and organize information based on the work done at the Institute of Agricultural Chemistry and Bacteriology at the College of Agriculture Berlin-Dahlen, as well as on data gathered elsewhere. The work is in the nature of a report on the progress of soil and plant research at the Experiment Stations at Berlin-Dahlen, Bonn, Bonn-Poppelsdorf, Braunschweig, the Peat Research Station at Bremen, the Agricultural Experiment Stations at Darmstadt, Jena, Königsberg, Landsberg, Lübeck, Münster, Oldenburg, and Weihenstephan.

To the student and investigator the data presented in this volume should be extremely helpful as a gauge of the progress of research in the fields of soil and plant science.

The Limestones and Marls of Minnesota. By CLINTON R. STAUFFER AND GEORGE A. THIEL. The University of Minnesota Press, Minneapolis, 1933. Pp. x + 193, figs. 93.

The authors note that:

> Minnesota has extensive deposits of calcium carbonate in the form of limestones, dolomites, and marls. This report deals primarily with the distribution and chemical composition of these carbonate deposits and the uses for which they are suited.

There is an introductory paragraph on "The Uses of Limestone, Dolomite, and Marl." Part I deals with "The Limestones and Dolomites of Minnesota," and Part II with "The Marls of Minnesota." Information is given in Part I on the nature and origin of limestone and dolomite, and of limestones and dolomites in individual counties. In Part II the authors discuss the nature and origin of marl and the relation of marl deposits to different types of glacial drift, the prospecting for marl, and marl deposits in individual counties. The illustrations and maps are well done. There is also a satisfactory index. The student of soils and allied subjects might well add this volume to his reference shelf.

Agricultural Systems of Middle Europe. Edited by O. S. MORGAN. The Macmillan Co., New York, 1933. Pp. xix + 405, 8 maps.

We may well quote former Secretary of Agriculture Arthur M. Hyde when he says, in the Foreword,

> The time has long since passed when the American farmer could afford to ignore what farmers in other parts of the world were doing. American agriculture is not a separate but an integral part of the world's economic system, and it is always deeply affected by financial, industrial and social conditions both at home and abroad.

The book deals with the author's studies and observations in some of the European countries. To use his own words,

> This book attempts to contribute authentic agricultural economic summaries of national agricultural programs and policies of Central European states. It is the combined result of the labors of busy agricultural officials and the editor. It is hardly the composite voice of the respective nationals, but it represents fairly, with a minimum of political and nationalistic effusion, the voices of authoritative spokesmen.

He says, further,

> This symposium is not designed as a textbook for students of agriculture and agriculture economics. Rather it is offered as a source book for agriculturists, economists, politicians and other students of economic and social phenomena. It is hoped it will serve as valuable collateral reading in college courses devoted to the study of national, agricultural, economic and political policies.

The contents of the book are made up of a Foreword by Arthur M. Hyde; an Introduction by O. S. Morgan; The Austrian Agrarian Policy, by Leopold Hennet and Anton Steden; Bulgarian Agriculture, by J. S. Molloff; Czechoslovak Agriculture, by Vladislav Brdlik; The Agricultural Policy of Greece, by Georges Servakis and C. Pertountzi; Agriculture and the Agricultural Economic Policy of Hungary, by Ivan Edgar Nagy; Polish Agriculture, by Waclaw Ponikowski and Victor Lesniewski; Aspects of Rumanian Agriculture, by A. Frundianescu and G. Ionescu-Sisesti, and The Economic Position and Future of Yugoslavian Agriculture, by Velimir N. Stoykovitch. The editor and his collaborators have rendered a useful service.

Agricultural Russia and the Wheat Problem. By VLADIMIR P. TIMOSHENKO. Food Research Institute and the Committee on Russian Research of the Hoover War Library, Stanford University, California. Pp. xi + 571, maps 4, charts 10, tables 54.

This book is the first of the publications to be included in the Grain Economics Series published by the Food Research Institute.

The authors tell us that:

> The purpose of this study is to analyze present conditions of Russian agriculture, particularly grain production, in the light of pre-war developments. Without study of the historical background, it is hardly possible to form an independent and well-founded judgment of conditions as they are now. More than one publication dealing with economic conditions in the USSR, and especially those written by visitors to Russia, suffer from lack of historical perspective.

He says further:

> Analysis of Russian agricultural conditions could not be confined strictly to presentation and interpretation of statistics. Soviet policy toward agriculture, especially collectivization and state farms, was too important a factor to be passed over even though it be, for the most part, not amenable to quantitative treatment. In this part of the discussion, I have made use of articles published currently in Russia, of course using sources critically but substantiating any criticisms with direct quotations from publications under the control of the Soviet government or the Communist party.

The book contains 16 chapters, appendix tables, and an index. The chapters deal, respectively, with Land, Climate and Population; Land Utilization and the Outlook for Expansion of Crop Area; Distribution of Landed Property and the Agrarian Revolution; Soviet Agricultural Policy; Collective and State Farms; The Development of Agricultural Production in Russia; Agricultural Organization and Practices; Cultivation of the Bread Grains; Bread-Grain Yields per Acre; Characteristics of Winter Wheat; Transportation, Storage and Milling; Crops and their Disposition; Domestic Shipments by Rail and Water; Post-War Grain Collections; Exports of Bread Grain and Flour; and The Outlook. There is much in this book of interest and value to the economist, agronomist, and statesman. It constitutes, therefore, a worth while addition to our list of books on Soviet Russia.

Forestry—An Economic Challenge. By ARTHUR NEWTON PACK. The Macmillan Company, New York, 1933. Pp. 161.

Our public domain constituted at one time an empire of a billion acres. Much of it was covered with forests, which served as a major factor in the development of the United States. Much of this great resource has been destroyed. As a result of this destruction, there have arisen most serious problems of flood control, soil erosion, and crop surpluses. We have now come to the point of rebuilding our forests. The reading public will find "Forestry: An Economic Challenge" a particularly timely contribution to an important subject.

The reader will readily agree with the author when he says:

> Men who call themselves foresters will have to examine themselves and ask pertinent—even impertinent—questions. There are some in the forestry profession, experienced men and new radicals alike, who are inclined to belittle the idle-land problem, to discount the values to be obtained through reforestation and to point to the tremendous unsolved problems of the growing and potentially productive forests which we still have, as more important. These problems are indeed tremendously acute. We have bungled them thoroughly enough, but they still remain the questions that must be solved first. Why talk about producing more forests when we have a lumber industry flat on its back, suffocated with the burden of manufacturing and selling the timber and pulpwood now available? How can foresters lead on to wider horizons when they are themselves completely lost in the woods?

The six chapters of the book deal, respectively, with Re-thinking Forestry; From Crusade to Economic Problems; Forest Use; Private Enterprise; Public

Forest Administration; Foresters and Forest Education, and Regional Planning and Economic Policy.

Forestry Almanac, 1933 Edition. Compiled and edited by THE AMERICAN TREE ASSOCIATION, Washington, D. C., 1933. Pp. xv + 484.

The book contains a discussion of the United States Forest Service, national forests, other government forestry activities, national forestry legislation, forests in our Territories, government reorganization, United States Timber Conservation Board, our forest inventory, Roosevelt on forestry, forest employment survey, American lumber industry, lull in the development of commercial forests, demonstration forests, forestry in varied fields, American trees rebuilding European forests, Charles Lathrop Pack Forestry Foundation, national forestry organizations, regional forestry associations, state forestry associations, cooperative organizations, foreign forestry organizations, forestry education, Charles Lathrop Pack Forest Education Board, forestry schools, state facts and statistics, forests and taxation, Canada and her forests, forestry in foreign countries, national park service, state parks, Bicentennial tree planting, how to plant trees, what trees to plant, arbor days in each state, trees with famous names, associations of forest industries, private technical foresters, and forestry books.

In discussing the progress of forestry, Charles Lathrop Pack, the President of the American Tree Association, says:

> With increasing clearness the past three years have brought out new and enlightening trends in the field of forestry. It is significant that this trend has been ever in the direction of greater realism and of clearer insight into the part forestry must ultimately play in the economy of the nation. Some familiar bugaboos have been laid to rest. For twenty-five years we have been hearing much of an approaching timber famine—a day when the last tree would crash to earth beneath this woodman's ax and we should look in vain for more. Recent studies during the present year show that the threat of any immediate failure in our wood supply is baseless, but they emphasize the unsatisfactory distribution of our commercial forests, with its attendant burden of high transportation charges necessary to bring forest products over long distances to the centers of consumption. The progressive destruction of our forests through fire and uneconomic cutting is still very real.

The information contained in the Almanac is almost indispensable for every student of our forests and land resources. The sociologist and the statesman will find in it much timely and helpful information.

Game Management. By ALDO LEOPOLD with drawings by Allan Brooks. Charles Scribner's Sons, New York-London, 1933. Pp. xxi + 481, figs. 35, tables 53.

The author defines game management as "the art of making land produce sustained annual crops of wild game for recreational use." In view of the growing interest in the decentralization of industry and population, the development of recreational facilities, and particularly the conservation of our land

and forests, game management becomes a topic of major interest to the public at large.

This book consists of three parts; namely, Part I. Management Theory; Part II. Management Technique; and Part III. Game Administration. There is also an appendix made up of a bibliography and glossary of terms used in game management, breeding potential tables, and index. The 18 chapters deal, respectively, with: I. A History of Ideas in Game Management; II. Mechanism of Game Management; III. Properties of Game Populations: Fluctuation and Density; IV. Properties of Game Populations; Movements, Tolerances, and Sex and Flock Habits; V. Game Range; VI. Measurement of Game Populations; Game Census; VII. Measurement and Diagnosis of Productivity; VIII. Game Refuges; IX. Control of Hunting; X. Predator Control; XI. Control of Food and Water; XII. Control of Cover; XIII. Control of Disease; XIV. Accidents; XV. Miscellaneous Techniques; XVI. Game Economics and Esthetics; XVII. Game Policy and Administration; XVIII. Game as a Profession. The illustrations and bibliography add substantially to the value of the work.

J. G. LIPMAN.

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